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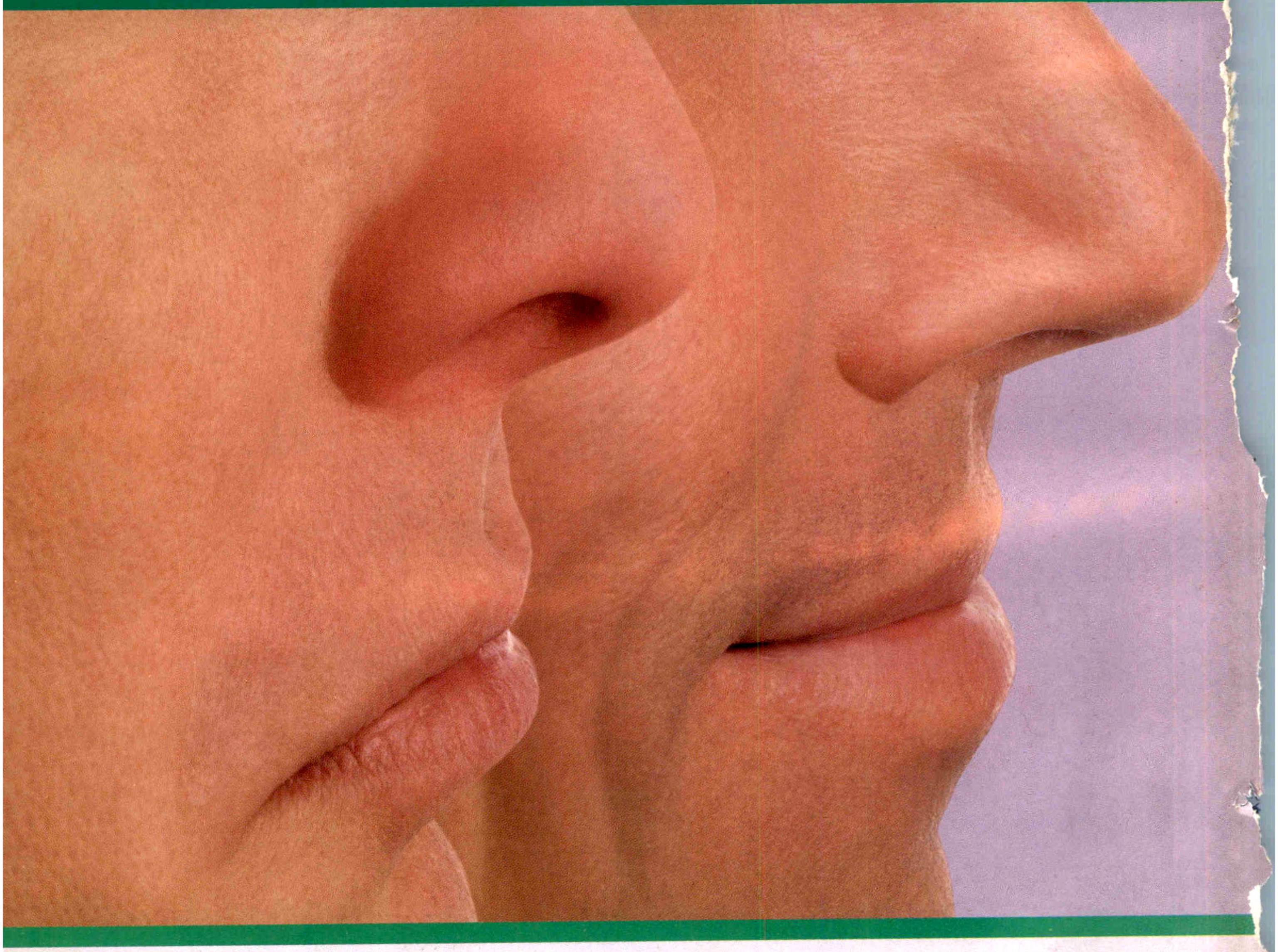

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VOLUME 105 JULY 1996 NUMBER 7

CONTENTS

FROM THE AMERICAN LARYNGOLOGICAL ASSOCIATION

Long-Term Follow-up of Tracheoesophageal Puncture Results
James A. Geraghty, MD; Barry L. Wenig, MD, MPH;
Bonnie E. Smith, PhD; Louis G. Portugal, MD 501

Acoustic Measurement of Subglottic Stenosis
Judith M. Czaja, MD; Thomas V. McCaffrey, MD, PhD 504

FROM THE AMERICAN BRONCHO-ESOPHAGOLOGICAL ASSOCIATION

Nd:YAG Laser Treatment for Laryngeal and Hypopharyngeal Hemangiomas: A New Technique
Seth A. Yellin, MD; Anthony LaBruna, MD; Vijay K. Anand, MD 510

Evaluation and Management of Hemoptysis in Infants and Children. A Report of Nine Cases
Jerome W. Thompson, MBA, MD; Chi D. Nguyen, MD; Rande H. Lazar, MD;
Rose Mary Stocks, PharmD, MD; Robert A. Schoumacher, MD;
Firas Hamdan, MD; K. Van Nguyen, MA, MD 516

Repeatedly Successful Closure of the Larynx for the Treatment of Chronic Aspiration With the Use of Botulinum Toxin A
Claus A. Pototschnig, MD; Imma Schneider, MD;
Hans E. Eckel, MD; Walter F. Thumfart, MD 521

Immunohistochemical Characterization of Benign Laryngeal Lesions
Mark S. Courey, MD; Jack A. Shohet, MD;
Margie A. Scott, MD; Robert H. Ossoff, DMD, MD 525

Acute Airway Obstruction in the Hospitalized Infant: Four Hard Lessons in the Distal Trachea
Glenn Isaacson, MD 532

Omohyoid Muscle Transposition for the Treatment of Bowed Vocal Fold
Hisayoshi Kojima, MD; Shigeru Hirano, MD; Kazuhiko Shoji, MD;
Koichi Omori, MD; Iwao Honjo, MD 536

Management of Subglottic Foreign Bodies
David J. Halvorson, MD; Robert M. Merritt, MD;
Christopher Mann, MD; Edward S. Porubsky, MD 541

Videoendoscopic Analysis of Laryngeal Function During Laughter
Martin J. Citardi, MD; Eiji Yanagisawa, MD; Jo Estill, MA 545

Nitrergic Innervation of the Rat Larynx Measured by Nitric Oxide Synthase Immunohistochemistry and NADPH-Diaphorase Histochemistry
Yasuo Hisa, MD; Nobuhisa Tadaki, MD; Toshiyuki Uno, MD; Shinobu Koike, MD;
Masaki Tanaka, MD; Hitoshi Okamura, MD; Yasuhiko Ibata, MD 550

Pediatric Tracheobronchial Foreign Bodies: Historical Review From The Johns Hopkins Hospital
C. Anthony Hughes, MD; Fuad M. Baroody, MD; Bernard R. Marsh, MD 555

INDEPENDENT CONTRIBUTIONS

Adenovirus-Mediated Gene Therapy for Head and Neck Squamous Cell Carcinomas
Eric A. Goebel, MD; Beverly L. Davidson, PhD; Joseph Zabner, MD;
Scott M. Graham, MD; Jeffrey A. Kern, MD 562

Onlay Bone Augmentation With an Osteoinductive Implant
Hugh E. Hetherington, MD; Jeffrey O. Hollinger, DDS, PhD;
Michael R. Morris, MD; William R. Panje, MD 568

IMAGING CASE STUDY OF THE MONTH

Magnetic Resonance Imaging of an Inhaled Peanut
Hiroshi Kimura, MD; Shin Aso, MD; Masatsugu Asai, MD;
Hajime Nakagawa, MD; Yukio Watanabe, MD 574

CLINICOPATHOLOGICAL CONSULTATION

Laryngeal Lymphoma Derived From Mucosa-Associated Lymphoid Tissue
Hans-Peter Horny, MD; Alfio Ferlito, MD; Antonino Carbone, MD 577

MISCELLANEOUS

Letters to the Editor 584

Book Review 584

Information for Subscribers 4a

Instructions to Authors 7a

Index to Advertisers 16a

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LONG-TERM FOLLOW-UP OF TRACHEOESOPHAGEAL PUNCTURE RESULTS

JAMES A. GERAGHTY, MD
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Since its introduction by Blom and Singer in 1980, tracheoesophageal puncture with a voice prosthesis has become the most frequently recommended choice for speech rehabilitation of total laryngectomees. Many studies have reviewed the initial speech acquisition success rates following tracheoesophageal puncture; however, long-term follow-up in these initial successes has been lacking. In addition, factors predictive of long-term success with tracheoesophageal speech have not been defined. Over a 10-year period, we retrospectively reviewed all total laryngectomy patients, including those who have undergone primary or secondary tracheoesophageal puncture, at the University of Illinois Hospital and Clinics and the Westside Veterans Administration Hospitals. Survival in the total laryngectomy cohort of 202 patients ranged from 35% to 50%. Forty of these patients underwent tracheoesophageal puncture, in whom survival was 75%. Short-term success with tracheoesophageal speech was approximately 70% for our patients, while long-term success was achieved in 66%. Despite low socioeconomic status and relatively high alcoholism rates, successful maintenance of tracheoesophageal speech was achieved in the majority of cases. Tracheoesophageal speech should therefore be considered as a primary method of vocal rehabilitation in all patients undergoing total laryngectomy.

KEY WORDS — laryngectomy, rehabilitation, voice.

INTRODUCTION

Over the 14 years since its introduction by Singer and Blom[1] in 1980, tracheoesophageal puncture (TEP) has been proven relatively safe and effective as either a primary or secondary voice rehabilitation method.[2-6] Despite its overall success, concern remains among otolaryngologists and speech pathologists with regard to its long-term reliability,[7] and also with regard to its possible contraindication among alcoholic and less compliant patient populations.

Tracheoesophageal puncture has been performed since 1982 at the University of Illinois Hospital and Clinics and the Veterans Administration Westside Hospital. Based on this extended experience, a retrospective follow-up study was designed to examine the long-term success and survival of our patient cohort, as well as the possible effects of social and economic factors.

METHODS

During the 10-year period from January 1984 to April 1994, 202 patients underwent total laryngectomy at the University of Illinois Hospital and Clinics (Illinois Eye and Ear Infirmary) and the Veterans Administration Westside Hospital. This cohort consisted of 167 men and 35 women.

Total laryngectomy was performed for T3 or T4 squamous cell carcinomas, combined with partial pharyngectomy, neck dissection(s), or postoperative radiotherapy as indicated. Most patients also routinely underwent cricopharyngeal myotomy, which is now standard practice at our institution.

Tracheoesophageal puncture, utilizing the Blom-Singer prosthesis, was carried out on 40 of these patients, of whom 30 were men. Their average age was 56 years (range 38 to 70). Primary tracheoesophageal puncture, at the time of laryngectomy, was performed on 18 patients. Selection of candidates was based on the patients' preoperative desire for voice rehabilitation, and informed consent. Secondary TEP was performed on 22 patients, with 14 months as the average interval between laryngectomy and puncture. Selection of these candidates was based on the patient's desire for TEP and the ability to cooperate with the speech pathology preoperative evaluation, as well as physical prerequisites such as adequate visual acuity and manual coordination. On the basis of insufflation testing results, several patients underwent myotomy at the time of secondary puncture.

Between May 1984 and October 1994, the charts for all 40 puncture patients were reviewed. Twenty-nine of these patients were alive and receiving follow-up care; 27 of these 29 were examined by an otolaryngologist, and answered a series of questions from the otolaryngologist and speech pathologist.

Short-term success was defined as initial acquisi-

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Presented at the meeting of the American Laryngological Association, Palm Desert, California, April 29-30, 1995.

TABLE 1. SURVIVAL IN TOTAL LARYNGECTOMY COHORT

	No.	*%*
Disease-free survival	71	35
Lost to follow-up	30	15
Deceased	99	49
Known recurrence or second primary	2	1
Total	202	100

tion of intelligible tracheoesophageal speech. Successful TEP use, on long-term follow-up, was defined as "using TEP for majority of verbal communication needs," and required speech to be intelligible to the examiners. Use of alternative modes of speech was also evaluated to verify that the TEP was, in fact, the primary mode.

RESULTS

Initial evaluation of the entire laryngectomy cohort (Table 1) revealed a survival rate of 35% to 50%. We presume that the majority of those patients "lost to follow-up" are probably deceased.

Forty patients, with an average age of 56 years, underwent TEP. Survival was approximately 75%, and initial voice success 70% (Table 2). Three of the initial 12 failures were due to either medical problems or surgical complications of laryngectomy in the postoperative period, preventing either use of the puncture or training. Twenty-nine of the 40 TEP patients are currently alive and receiving follow-up care, with a long-term TEP success rate of 66%. We were able to perform a follow-up interview in 27 of the 29 available patients. The average length of follow-up for this group was 39.3 months.

The patients' educational levels (Table 3) were low but did not affect TEP success, with the exception of a slight trend toward failure with higher levels of education. Evaluation of patients' economic standing (Table 4) reflected their largely lower-class status, but similar to education, this did not seem to detract from their chances for success.

TABLE 2. SURVIVAL AND VOICE SUCCESS IN TEP PATIENT COHORT

	No.	*%*
Initial evaluation		
Initial success (primary TEP)	12/18	67
Initial success (secondary TEP)	16/22	73
Initial success (total)	28/40	70
Follow-up evaluation		
Deceased	10/40	25
Lost to follow-up	1/40	2.5
Long-term success	19/29	66

TEP — tracheoesophageal puncture.

TABLE 3. EDUCATION AND VOICE SUCCESS

	*TEP Result**		
	Early Failure	*Early Success*	*Late Success*
Eighth grade or less	1/27	8/27	8/18
Ninth through 12th grade	6/27	9/27	8/18
College	2/27	1/27	1/18

*$p > .10$ for entire group (failure-success ratios compared among all three educational groups); $p = .036$ for only eighth grade versus college.

Only 2 patients in the follow-up group admitted to recreational drug use. Alcoholism (Table 5), by comparison, was present in 18 of 27 patients prior to TEP. The majority of alcoholics had quit drinking by the postoperative period. Among those who continued with heavy alcohol use postoperatively, 4 out of 6 maintained good tracheoesophageal speech.

For comparison of speech success among the subgroups in each of Tables 3, 4, and 5, a Fisher exact test was used. The results are footnoted on each Table. The differences in speech success only achieved statistical significance in comparison of those patients with an eighth grade education versus those with a college education ($p = .036$).

No operative or immediate postoperative complications were noted in the 40 TEP patients, although 2 eventually required repeat TEP because of difficulty with prosthesis placement or position. Of the 27 patients with long-term follow-up, the following were noted: 4 episodes of difficulty with insertion of prosthesis, 3 episodes of stomal stenosis, 1 aspiration of the prosthesis, and 2 episodes of fistula leakage (8 patients total). There were no fatalities or serious morbidities attributable to the TEP.

DISCUSSION

Tracheoesophageal puncture appears to have gained acceptance as a relatively safe and effective method of voice restoration,[2-6] and has even been performed

TABLE 4. ECONOMIC STATUS

		Early Failure	*Early Success*	*Late Success*
Preoperative maximum yearly income	<\$10,000	4/27	9/27	9/18
	\$10,000-20,000	2/27	7/27	7/18
	>\$20,000	3/27	2/27	1/18
Postoperative yearly income	<\$10,000	7/27	17/27	16/18
	\$10,000-20,000	1/27	1/27	1/18
	>\$20,000	1/27	0/27	0/18

$p > .42$ for preoperative income group, $p = .25$ for postoperative income group, where p compares failure-success ratios among three income subgroups.

TABLE 5. ALCOHOL USE AND VOICE SUCCESS

	Early Failure	*Early Success*	*Late Success*
No history of alcoholism	5/27	4/27	3/18
History of alcoholism (total)	4/27	14/27	14/18
Quit months or years prior to TEP	1/27	3/27	3/18
Quit at time of TEP	1/27	7/27	7/18
Continued heavy alcohol use	2/27	4/27	4/18

$p > .36$ for entire group, $p > .20$ for only abstaining and heavy use groups, where p compares failure-success ratios among rows 1, 2, and 5.

under topical or local anesthesia.[8,9] Despite this acceptance, however, many patients are still not rehabilitated. Possible reasons for this include selection bias,[7] recurrent carcinoma, and refusal of rehabilitation.[5] A portion of the selection bias has consisted of patients not being considered for puncture because of perceived inability to comply with training or care for the prosthesis.

The screening process for our patients with primary punctures consisted only of vision evaluation and consent for the procedure; screening for secondary procedures consisted of the patient compliance with three preoperative visits and esophageal insufflation evaluation, as well as visual acuity.

Our patients generally had low levels of formal schooling and income, and a high rate of alcoholism. Despite these factors, which are felt to predispose to patient noncompliance and failure, our patients demonstrated adequate (70%) success, and maintained this surprisingly well (66%) over an average follow-up period greater than 3.5 years.

A reasonable conclusion, based on this, is that patient motivation alone should provide the basis for selection of patients for TEP, regardless of education, economic status, or substance abuse. Previous studies[5,7] have shown that in an unselected series of laryngectomy patients, up to 26% or more simply refuse or do not follow through with voice restoration.

CONCLUSION

The success of tracheoesophageal speech acquisition in laryngectomy patients has been adequately demonstrated elsewhere. Our series shows that long-term maintenance of this success is possible, even in an urban population of low socioeconomic standing with high alcoholism rates.

The superior quality of tracheoesophageal speech,[10-12] as well as the ease of performance at the time of laryngectomy, makes TEP a good choice for laryngectomee rehabilitation. This option should be presented to all patients physically able to care for and use the prosthesis.

REFERENCES

1. Singer MI, Blom ED. An endoscopic technique for restoration of voice after laryngectomy. Ann Otol Rhinol Laryngol 1980;89:529-33.
2. Blom ED, Singer MI, Hamaker RC. A prospective study of tracheoesophageal speech. Arch Otolaryngol Head Neck Surg 1986;112:440-7.
3. Wenig BL, Mullooly V, Levy J, Abramson AL. Voice restoration following laryngectomy: the role of primary versus secondary tracheoesophageal puncture. Ann Otol Rhinol Laryngol 1989;98:70-3.
4. Maniglia AJ, Lundy DS, Casiano RC, Swim SC. Speech restoration and complications of primary versus secondary tracheoesophageal puncture following total laryngectomy. Laryngoscope 1989;99:489-91.
5. Lacau St Guily J, Angelard B, El-Bez M, et al. Postlaryngectomy voice restoration. Arch Otolaryngol Head Neck Surg 1992;118:252-5.
6. Kerr AIG, Denholm S, Sanderson RJ, Anderson SJ. Blom-Singer prostheses — an 11 year experience of primary and secondary procedures. Clin Otolaryngol 1993;18:184-7.
7. Gates GA, Ryan W, Cooper JC, et al. Current status of laryngectomee rehabilitation: I. Results of therapy. Am J Otolaryngol 1982;3:1-7.
8. Maniglia AJ. Voice rehabilitation after total laryngectomy: a flexible fiberoptic endoscopic technique for tracheoesophageal fistula. Laryngoscope 1982;92:437-9.
9. Gross M, Hess M. A new method for tracheoesophageal puncture under topical anesthesia. Laryngoscope 1994;104:233-4.
10. Williams SE, Watson JB. Differences in speaking proficiencies in three laryngectomy groups. Arch Otolaryngol 1985;111:216-29.
11. Williams SE, Watson JB. Speaking proficiency variations according to method of alaryngeal voicing. Laryngoscope 1987;97:737-9.
12. De Maddalena H, Pfrang H, Schohe R, Zenner HP. Speech intelligibility and psychosocial adaptation in various voice rehabilitation methods following laryngectomy [in German]. Laryngorhinootologie 1991;70:562-7.

Ann Otol Rhinol Laryngol 105:1996

ACOUSTIC MEASUREMENT OF SUBGLOTTIC STENOSIS

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A device that determines cross-sectional area (CSA) of the airway by acoustic reflections (Hood, Inc) was used to measure subglottic area. Airway models were made from Plexiglas rings with known internal dimensions similar to clinically encountered stenoses of various lengths and diameters. Acoustic measurements of airway area were made and compared to actual CSA. There is a strong correlation between CSA measured acoustically and the actual area of simulated stenoses. However, when the CSA of the stenosis was <0.64 cm^2, the signal was impaired, resulting in overestimation of the stenotic CSA. In simulated stenoses with a CSA of <0.38 cm^2, acoustic measurement of the CSA beyond the stenotic segment was unreliable. Determination of the origin of stenosis was accurate with this method. The CSA of cadaver airways was also measured acoustically. The CSA 2.0 cm below the glottis of normal airways in males ranged from 1.28 to 2.74 cm^2 and in females 0.87 to 1.43 cm^2, with means of 2.16 and 1.09 cm^2. It appears that acoustic measurement of CSA of subglottic stenosis is a feasible clinical technique that yields dimensions of the airway in situations in which direct measurements are impossible. It was suggested that this technique be used for assessment of subglottic stenosis and evaluation of the efficacy of treatment of subglottic stenosis.

KEY WORDS — acoustics, laryngeal stenosis, tracheal stenosis.

INTRODUCTION

The use of acoustic impulse reflection to measure airway cross-sectional area (CSA) has been an established investigational technique since its introduction by Jackson et al[1] in 1977. Cross-sectional area as a function of distance along the airway can be determined by analysis of a reflected acoustic signal. Primarily utilized to measure total CSA of the distal (lower) trachea and lower airways, this technique correlates strongly with actual CSA measurements at specified distances along the tracheobronchial tree. Human and animal studies have been performed to demonstrate this correlation. Techniques used to examine actual airway CSA for comparison with acoustic measurement have included air bronchograms, plain chest radiographs (anteroposterior and lateral), and sectioning of negative silicone rubber casts of the tracheobronchial tree.[2-5] The accuracy of cross-sectional measurements made by the acoustic method has been demonstrated to the level of fifth-generation bronchial airways.[4] Although the technique of acoustic impulse reflection in the tracheobronchial tree has played a significant role in investigational studies, its use as a diagnostic and prognostic test in the clinical setting has been limited. Only one study to date has been performed to investigate the utility of this technique in the clinical diagnosis of upper airway obstruction.[6]

Although the technique was initially designed to examine the tracheobronchial tree, Jackson's[1] original system has been adapted for clinical use in the measurement of the CSA of the nasal airway. Acoustic rhinometry was first described by Hilberg et al[7] in 1989. It is based on acoustic principles identical to those applied to the measurement of total CSA of the central airway, namely, 1) unidimensional wave propagation, 2) negligible thermal and viscous losses, 3) symmetric and regular bifurcation of airstreams, and 4) maintenance of airway wall rigidity over the frequency range of the acoustic impulse.[5] The technique of acoustic rhinometry in the management of nasal obstruction has proved effective in the diagnosis of structural and mucosal abnormalities. Furthermore, it is valuable in the postoperative evaluation of rhinosurgical procedures. The commercial availability of acoustic measuring devices for rhinometry now makes this application to the laryngotracheal airway possible.

The evaluation of laryngotracheal stenosis is limited by the lack of standardized methods for estimation of the total CSA and length of the stenotic segment. Current methods for the diagnosis and evaluation of laryngotracheal stenosis include plain radiography, tracheal tomograms, computed tomography (CT) volume reconstructions, magnetic resonance imaging (MRI), pulmonary function tests, and direct laryngoscopy. Although these diagnostic tests are able to qualitatively evaluate the severity of laryngotracheal stenosis, they do not provide accurate measurements of true CSA. Use of CT and MRI is expensive and time-consuming, and only yields accurate measurements of CSA when the plane of

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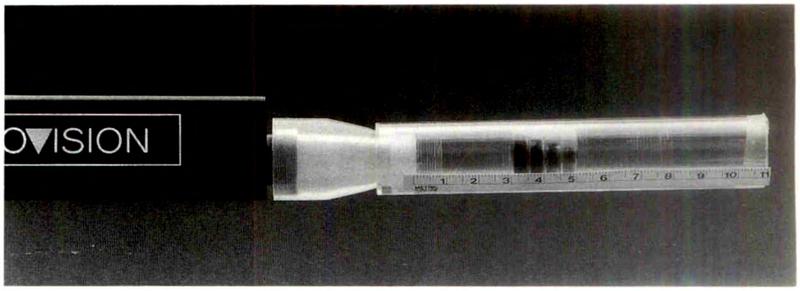

Fig 1. Acoustic wave tube fitted with silicone nosepiece and Plexiglas tube. Nosepiece tip marks level of glottis at 0 mm.

study is perpendicular to the airway. Direct laryngoscopy is subjective when describing the stenosis as a percentage of the total CSA, and is inaccurate when evaluating length of stenotic segments. The ideal method used to diagnose and evaluate laryngotracheal stenosis should be simple, rapid to perform, and inexpensive, pose no increased risk to the patient, and be conveniently adapted for use in the office setting.

The technique of acoustic impulse reflection to assess upper airway obstruction by measurement of total CSA and length of stenotic segments is feasible. The method has proven effective and accurate in the determination of CSA in previous studies of nasal and central airways. The purposes of this study are to compare the actual CSA with the acoustically measured CSA of simulated laryngotracheal stenoses, to determine the accuracy of acoustic impulse reflection in the measurement of these stenoses, and to use acoustic impulse reflection to provide standardized measurements of subglottic CSA of 20 human cadaver larynges without a previous history of laryngotracheal disease.

MATERIALS AND METHODS

Acoustic Rhinometer. The acoustic rhinometer was easily adapted for our investigation of simulated laryngotracheal stenoses and cadaver larynges. The equipment and theory of acoustic rhinometry have been previously described by many investigators.[7,8] The system consists of a computer (desktop PC), software provided by Hood, Inc, an acoustic tube (Eccovision) equipped with a microphone, a click generator, and a calibration tube attachment.

A click filtered between 150 Hz and 10 kHz generates the initial acoustic impulse that propagates along the wave tube. This impulse passes a microphone that receives the incident wave, then travels through an attached nosepiece of known diameter fitted to the artificial larynges. The acoustic impulse passes into the attached Plexiglas tube and is reflected at the step discontinuities representing changes in CSA of the artificial laryngotracheal segments. Reflected analog wave signals are received by the microphone, amplified, low-pass filtered, and digitized. The software converts the acoustic data to area-distance functions that are plotted on a logarithmic scale. The sampling time for each trial is approximately 1.5 seconds per impulse.

Calibration of the system prior to experimentation involved attachment of a calibration tube of constant diameter to the acoustic tube in order to calculate the incident acoustic wave. All subsequent measurements of reflected acoustic waves were calculated by subtraction of the calibrated incident wave from the total incident and reflected waves received by the microphone in the acoustic tube. Calibration was performed daily at the initiation of the experimental trials.

Model Studies. Plexiglas tubes were fashioned to simulate male (large) and female (small) larynges. The large tube inner diameter measured 19 mm, corresponding to a CSA of 2.83 cm^2. The small tube inner diameter measured 13 mm, corresponding to a CSA of 1.33 cm^2. A series of Plexiglas rings were fashioned to fit the large and small tubes. The large rings had an outer diameter of 18 mm, and the inner diameters measured 9 mm, 7 mm, 5 mm, and 3 mm. The small rings had an outer diameter of 12 mm, and the inner diameters measured 7 mm, 5 mm, and 3 mm. Each of the rings in both groups was 5 mm in thickness. One end of each Plexiglas tube was securely fitted with a silicone nosepiece (Hood, Inc). Visualizing the end of the nosepiece through the clear Plexiglas tube, a millimeter measuring strip was affixed to the outside of the tube where 0 mm marked the tip of the nosepiece. The acoustic wave tube was calibrated and the nosepiece–Plexiglas tube was attached (Fig 1). A variety of stenoses were created by using the Plexiglas rings of different sizes. These represented simple straight, hourglass, and conical tapering stenoses (Fig 2). These were placed into the Plexiglas tube and positioned to originate at various distances from the nasal tip as indicated by the millimeter ruler. In this model, the tip corresponded to the glottis; therefore, all measurements were referenced from this point. An acoustic impulse was generated by the acoustic rhinometer and an area-distance curve was calculated. Four area-distance curves of each stenosis model were obtained to test

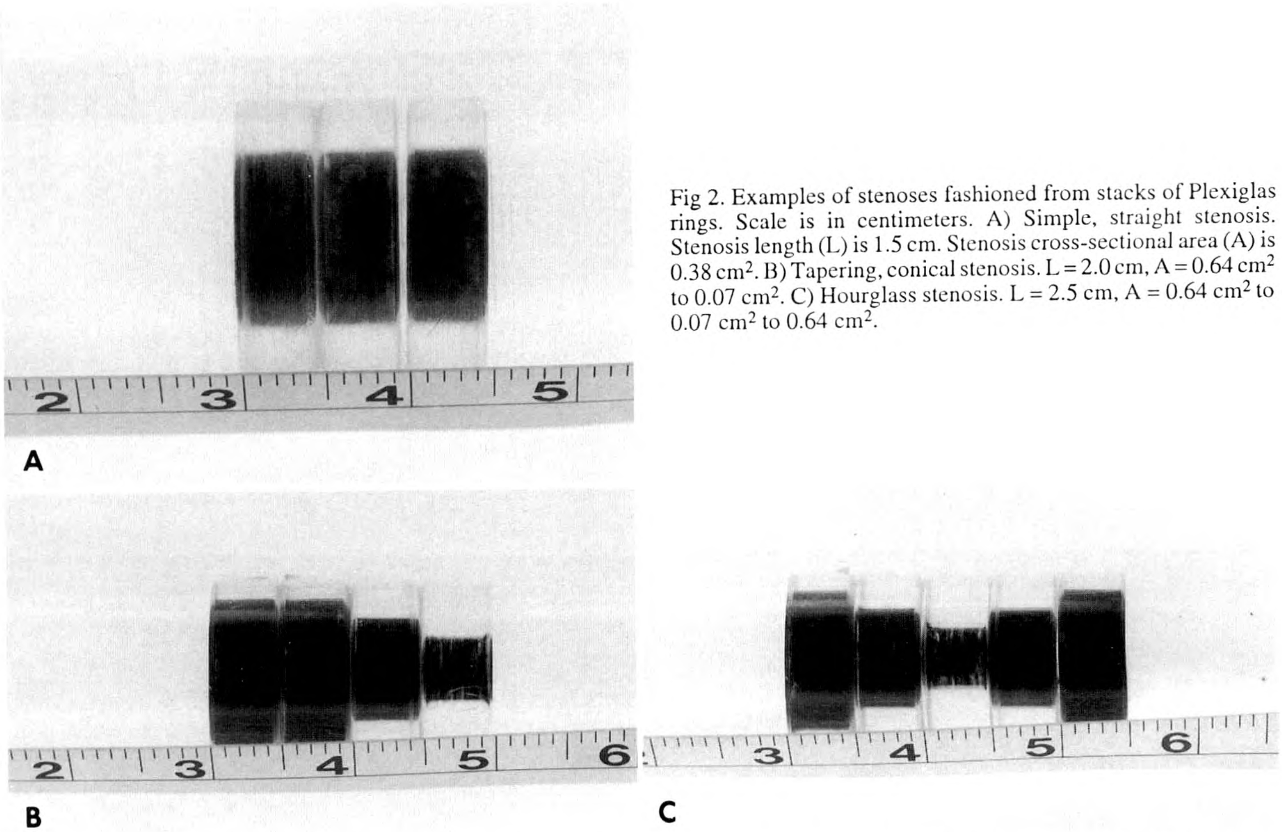

Fig 2. Examples of stenoses fashioned from stacks of Plexiglas rings. Scale is in centimeters. A) Simple, straight stenosis. Stenosis length (L) is 1.5 cm. Stenosis cross-sectional area (A) is 0.38 cm^2. B) Tapering, conical stenosis. L = 2.0 cm, A = 0.64 cm^2 to 0.07 cm^2. C) Hourglass stenosis. L = 2.5 cm, A = 0.64 cm^2 to 0.07 cm^2 to 0.64 cm^2.

the accuracy and reproducibility of the acoustic rhinometer. Each acoustic area-distance curve was plotted, and the actual area-distance curve (a step area discontinuity) was superimposed onto it for comparison by using the Sigma Plot graphing software (Jandel Scientific). Origin, length, and total CSA of the stenosis measured by the two techniques were compared.

Cadaver Studies. Twenty normal human cadaver larynges (10 male, 10 female) fixed in 10% formaldehyde were obtained from autopsy specimens. The epiglottis and preepiglottic fat, if present, were removed. Several centimeters of trachea were present on each larynx specimen, although the length of the tracheal remnants varied. All larynges were positioned on a securely fitting nosepiece attached to the acoustic wave tube so that the nosepiece tip was directly at the glottic level. An acoustic impulse was generated and an area-distance curve was calculated. Subglottic CSA measurements were made 2 cm below the glottis. The total length of each specimen was measured from the tip of the nosepiece to the end of the distal tracheal segment and recorded.

RESULTS

Laryngotracheal Models. With the tip of the nosepiece inserted into the artificial larynx at 0 mm to represent the glottic level, the origin of the stenosis was accurately determined acoustically. The acoustic wave began its negative deflection at a point prior to the origin of the stenosis, reaching the point of maximal negative slope of the acoustic deflection at the exact origin of the simulated stenosis (Fig 3A). This was reproducible in every trial, and this accuracy in measurement did not change as the distance of the stenosis origin increased from the tip up to 5.0 cm.

Acoustically determined CSA overestimated true CSA area (between 10% and 50%) in all cases. We found that by increasing the length of the stenotic segment, the acoustic CSA more closely approached the true CSA. In addition, increasing the diameter of the stenosis improved the accuracy of the acoustic CSA measurement (Fig 4).

Recovery of an accurate acoustic CSA measurement beyond the stenosis occurred variably. Poststenotic CSA was underestimated in all cases, and as the length of the stenotic segment increased, the acoustic poststenotic CSA less closely approximated the true CSA, with a gradual loss of the cylindric contour of the acoustic wave. Yet, as the area of the stenosis increased from 0.07 cm^2 to 0.64 cm^2 (corresponding to diameters of 3 and 9 mm, respectively), the true poststenotic CSA was more closely approximated acoustically (Fig 3B-E).

Termination of the stenotic segment was indicated

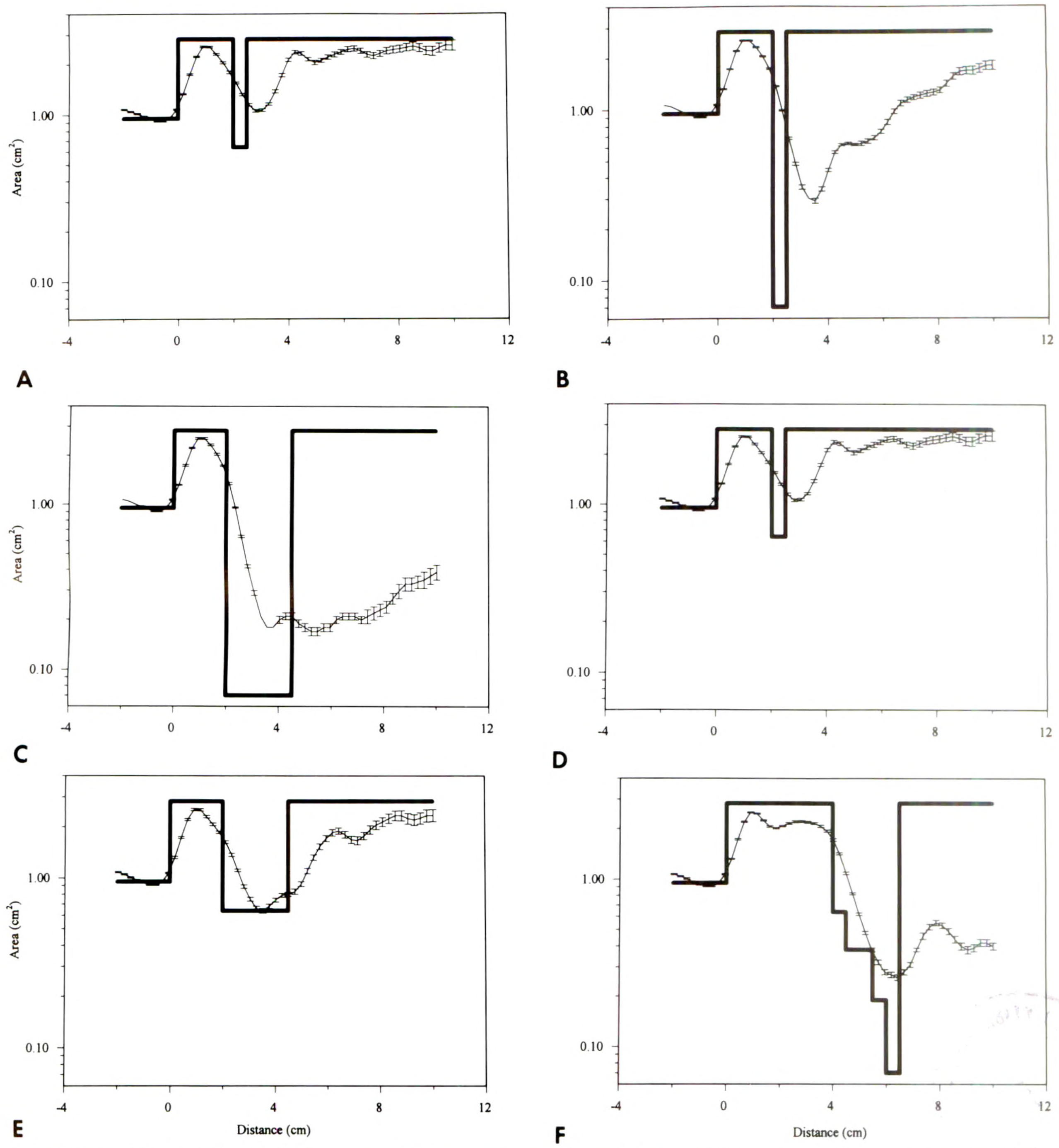

Fig 3. Area-distance curves. Bold line — true CSA, narrow line — acoustic CSA. A) Of simple, straight stenosis shown in Fig 2A. Origin and length of stenosis are accurately predicted by acoustic reflection. B) Of straight stenosis 0.5 mm long, 0.07 cm^2 total cross-sectional area (CSA). Short, narrow stenosis was less accurately measured by acoustic reflection. C) Of straight stenosis 2.5 cm long with identical CSA as in B. Total CSA measurement is more accurate as stenosis length increases. There is loss of accuracy in measurement of poststenotic segment in longer stenoses. D) Of straight stenosis 0.5 cm long, 0.64 cm^2 total CSA. This stenosis was more accurately measured by acoustic reflection than stenoses in B and C. E) Of straight stenosis 2.5 cm long, 0.64 cm^2 total CSA. When compared with B, this curve shows improved accuracy of acoustic reflection technique in longer stenoses with larger CSA. F) Of tapering, conical stenosis similar to that shown in Fig 2B. Acoustic damping occurs in prestenotic segment represented by bimodal peak in acoustic waveform. Subtle changes in tapering stenosis are not accurately detected by incident wave.

by the origin of the maximal slope of the acoustic wave after the curve minimum. This was a reliable indicator of stenosis length, even in the case of the most severe stenosis.

Cadaver Studies. The total CSA of human cadaver larynges was measured 2.0 cm below the level of the glottis in 10 male and 10 female larynges. In males, the CSA ranged from 1.28 cm^2 to 2.74 cm^2, with a

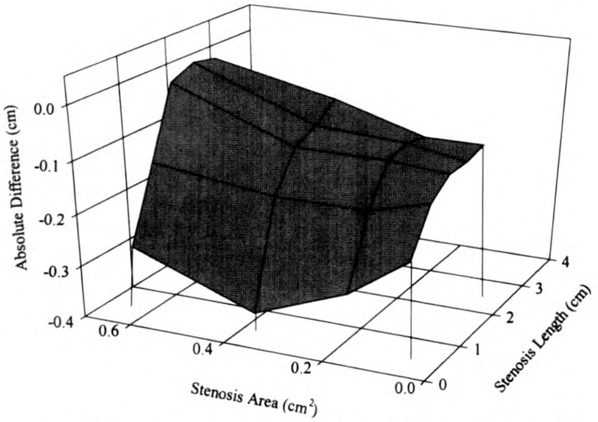

Fig 4. Three-dimensional curve shows absolute difference between measurement of acoustic wave CSA and true CSA. Absolute difference between two techniques approaches zero for longer stenoses of any CSA, and for stenoses with larger total CSA.

mean of 2.16 cm^2. This corresponds to a mean diameter of 1.65 cm. In females, the CSA ranged from 0.87 cm^2 to 1.43 cm^2, with a mean of 1.09 cm^2. This corresponds to a mean diameter of 1.17 cm.

DISCUSSION

The use of acoustic impulse reflection to measure airway dimensions has proved reliable, reproducible, and accurate.[1-5,9,10] When compared with conventional techniques available for the assessment of airway areas, acoustic techniques have been accurate to the level of fifth-generation bronchial airways.[4] Little has been done to utilize this technique in the clinical setting of laryngotracheal stenosis. Our objective in this study was to determine the accuracy of acoustic impulse reflection in the detection of upper airway narrowing.

The prestenotic acoustic CSA measurements closely approximated the step-area discontinuity representing true CSA in all cases. In those stenoses originating at a distance farther from the imaginary glottic level (nosepiece tip at 0 mm), a bimodal peak was seen. This occurred secondary to the summation of the incident wave and the reflected wave, which travels in a direction opposite to that of the propagating incident wave. This acoustic damping effect was maximal at larger distances and was negligible when the stenosis began at the origin of the tip (Fig 3F).

The origin of the stenotic segment was accurately predicted for all types of stenoses. The acoustic impulse was reflected at the initial step-area discontinuity and indicated the origin; however, for tapering, conical stenoses, subtle changes in the CSA from 0.64 cm^2 to 0.07 cm^2 were not accurately detected by the incident waves (Fig 3F). The sensitivity of the microphone receiving subtle reflections such as those occurring in a conical stenosis was poor. In addition, certain internal losses occur with acoustic wave propagation that also may limit this method in the detection of subtle changes, thus decreasing its accuracy.

These same considerations apply to the underestimation of the poststenotic segment CSA by acoustic reflection. Most of the incident wave energy was reflected at narrow stenoses; the remainder of the propagating waveform was therefore unable to closely approximate the true CSA beyond the stenosis. As we increased the diameter of the stenosis in our models, the estimation of the true CSA by the propagating wave improved. This has been shown by other investigators as well.[1] In addition, waves propagating through shorter stenoses were not attenuated as greatly as those traveling through longer, narrow segments; therefore, the approximation of the poststenotic CSA was more accurate. Despite the shortcomings of this technique in accurately predicting the poststenotic CSA, the length of the stenotic segment was clearly indicated in all trials. The first positive deflection of the acoustic wave after the stenotic origin correlated closely with the total length or termination of the stenotic segment. This was true in all cases, even those with the narrowest stenoses.

Certain acoustic principles of wave propagation must be assumed if acoustic impulse reflection is to be utilized to measure airway CSA. These theoretic considerations include unidimensional wave propagation, negligible thermal and viscous losses, regular branching bifurcation of airways, and rigid airway walls.[3,5] Whether the airway walls behave rigidly over the generated frequencies of propagating waves has been a disputed point in other studies analyzing CSA of in vivo and in vitro tracheobronchial segments.[2] Losses in a nonrigid wall by dissipation of propagating wave energy can lead to overestimations of the true CSA.[3,4] To increase airway wall rigidity in vitro, some investigators have coated (with moderate amounts of petroleum jelly) the tracheobronchial segments under study, thereby increasing wall inertance. Energy loss in these instances becomes negligible, and good estimates of CSA are obtained.[2] In vivo models have required different alterations in experimental technique to obtain increased airway wall rigidity. Dynamic airway segment wall compliance is altered by the frequency of the incident acoustic waves. At low frequencies (100 Hz), wall compliance is higher than the compliance of the surrounding air. As the incident frequency increases (100 to 1,000 Hz), wall compliance begins to decrease until, at frequencies greater than 1,000 Hz, airway segment wall inertia is maximal and dynamic compliance falls.[3] In an effort to maximize incident acoustic wave frequencies and energy, investigators have applied the principles of altered wave velocity in different compositions of gas.[2,3] Wave velocity in air is significantly less than in a mixture of 80% helium–20% oxygen (He-O_2; 34,300 cm/s versus 64,500 cm/s, respectively). By changing the medium through which incident waves propagate to He-O_2 mixtures, the frequency of an incident wave is increased to a point where dynamic airway wall compliance is negligible and airway walls are maximally rigid.[3] By allowing wave propagation in an He-O_2 mixture, incident wave velocity and frequency are increased, airway wall compliance is negligible, and the propagating wave energy is maximal, thereby improving estimated measurements of airway CSA.

Within the stenotic segment, the propagating wave overestimated CSA in all cases. The shorter segments of narrowest diameter yielded the poorest prediction of total CSA, as well as the termination of the stenosis. Our investigations were performed in room air. Perhaps one cause of overestimated stenotic CSA in our model was poor wave propagation in room air.

Limitations in the sensitivity of the acoustic system prevented accurate detection of rapid, small changes in CSA. The closest approximation of true CSA by acoustic reflection occurred in stenoses of CSA measuring 0.64 cm^2. These results suggest that the acoustic reflection technique with this system was less accurate in the evaluation of shorter stenoses with a CSA of less than 0.64 cm^2. When the length of the stenosis was increased, the incident propagating wave gradually conformed to the narrowed contour of the stenotic segment. This improved the estimation of total CSA for all diameters of model stenoses.

Cross-sectional areas measured acoustically have been compared with those measured radiographically or directly by positive and negative tracheobronchial tree casts. These comparisons have been used to demonstrate accuracy with the acoustic technique, rather than to define baseline values of acoustic CSA in normal male and female larynges. One study[11] comparing area measurements by acoustic impulse reflection versus CT scanning found the mean CSA by acoustic reflection to be 1.8 cm^2. This mean was calculated from measurements of 11 subjects who had histories of glottic disease and had received surgical therapy. The sex of the subjects included for study was not indicated. To our knowledge, the results we obtained in the acoustic measurement of larynges from 10 normal female and 10 normal male subjects without a previous history of laryngotracheal disease are the first standardized acoustic measurements of normal male and female laryngeal CSA below the glottis.

Acoustic measurement of CSA is a feasible clinical technique that can assist in diagnosis and evaluation of laryngotracheal stenosis. We have shown that the acoustic impulse reflection technique is reliable and reproducible for the measurement of simulated laryngotracheal stenoses when measurements are made within the limitations of the method ($CSA > 0.64\ cm^2$), yielding accurate results in our model studies.

Our protocol adapted the acoustic rhinometer for the model and cadaver studies. The limitation in our current setup is related to the size of the acoustic wave tube and the inability to utilize this at direct laryngoscopy. Modifications are being made to adapt the system for use at direct laryngoscopy and bronchoscopy. Further clinical trials are being performed to establish this technique for use in the assessment of patients with laryngotracheal stenosis and evaluation of the efficacy of treatments of laryngotracheal stenosis.

REFERENCES

1. Jackson AC, Butler JP, Millet EJ, Hoppin FG Jr, Dawson SV. Airway geometry by analysis of acoustic pulse response measurements. J Appl Physiol 1977;43:523-36.

2. Brooks LJ, Castile RG, Glass GM, Griscom NT, Wohl MEB, Fredberg JJ. Reproducibility and accuracy of airway area by acoustic reflection. J Appl Physiol 1984;57:777-87.

3. Fredberg JJ, Wohl MEB, Glass GM, Dorkin HL. Airway area by acoustic reflections measured at the mouth. J Appl Physiol 1980;48:749-58.

4. Jackson AC, Olson DE. Comparison of direct and acoustical area measurements in physical models of human central airways. J Appl Physiol 1980;48:896-902.

5. Jackson AC, Krevans JR. Tracheal cross-sectional areas from acoustic reflections in dogs. J Appl Physiol 1984;57:351-3.

6. Hoffstein V, Zamel N. Tracheal stenosis measured by the acoustic reflection technique. Am Rev Respir Dis 1984;130:472-5.

7. Hilberg O, Jackson AC, Swift DL, Pedersen OF. Acoustic rhinometry: evaluation of nasal cavity geometry by acoustic reflection. J Appl Physiol 1989;66:295-303.

8. Grymer LF, Hilberg O, Elbrond O, Pedersen OF. Acoustic rhinometry: evaluation of the nasal cavity with septal deviations, before and after septoplasty. Laryngoscope 1989;99:1180-7.

9. Jackson AC. Serial distribution of airway geometry from acoustic impedance data. Fed Proc 1980;39:2741-6.

10. Hoffstein V, Fredberg JJ. The acoustic reflection technique for non-invasive assessment of upper airway area. Eur Respir J 1991;4:602-11.

11. D'Urzo AD, Rubenstein I, Lawson VG, et al. Comparison of glottic areas measured by acoustic reflections vs. computerized tomography. J Appl Physiol 1988;64:367-70.

Ann Otol Rhinol Laryngol 105:1996

ND:YAG LASER TREATMENT FOR LARYNGEAL AND HYPOPHARYNGEAL HEMANGIOMAS: A NEW TECHNIQUE

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The treatment of laryngeal and hypopharyngeal hemangiomas is indicated when they are symptomatic, causing dysphagia, recurrent bleeding, or airway obstruction. These tumors are found in the glottis, supraglottic larynx, and hypopharynx. Histologically, they are considered as mixed or cavernous-type hemangiomas. By utilizing a glass slide to compress the lesion, its thickness and blood flow are reduced. The tumor can then be laser-photocoagulated with less energy, with the glass slide used as a laser platform. These conditions optimize the benefits of laser ablation while minimizing the adverse heat sink effects to the surrounding healthy tissue. We present three patients with hemangiomas of the larynx and hypopharynx who were treated with this technique. The details of the technique in each case will be presented with the objective of improving the care of these unusual and challenging tumors.

KEY WORDS — hemangioma, hypopharynx, neodymium:yttrium-aluminum-garnet laser.

INTRODUCTION

Laryngeal and hypopharyngeal hemangiomas are uncommon neoplasms that can cause dysphagia, recurrent bleeding, and airway obstruction. Historically, these lesions have been a therapeutic challenge and surgical resection has been the mainstay of therapy.[1,2] Since the introduction of the carbon dioxide laser in otolaryngology, laser therapy for vascular lesions has proven to be a very useful adjunct in the management of these vascular neoplasms.[3-7]

Laryngeal and hypopharyngeal hemangiomas have been divided clinically into pediatric and adult types.[8] Pediatric hemangiomas most often present as a subglottic mucosal-covered mass and are histologically of the capillary type with tangled nests of small blood vessels. Adult hemangiomas are usually found in the hypopharynx and larynx. Histologically, they are usually of the cavernous or mixed type, with a thin, friable mucosa overlying the vascular stroma. Others[9] have proposed that vascular lesions be divided into two groups, "hemangiomas" and "malformations," based on their degree of mitotic activity and postnatal growth characteristics. The nomenclature of vascular lesions remains controversial, and for the purposes of this article we will refer to them as hemangiomas and will limit our discussion to the adult type.

The neodymium:yttrium-aluminum-garnet (Nd:YAG) laser has several characteristics that make it well suited to the treatment of low-flow vascular lesions of the hypopharynx and larynx.[7] It produces a coherent beam of infrared radiation at a wavelength of 1,064 nm (invisible, near infrared) and can be carried by a flexible fiberoptic delivery system. The tissue characteristics of the Nd:YAG laser result in deep penetration (up to 1 cm in depth)[6] and scatter of laser energy that is preferentially absorbed by pigmented or vascular tissue. This allows photocoagulation of deeper structures while only minimally disrupting the overlying mucosa.[10] The rapid regenerative capacity of the mucosa and judicious selection of laser energy parameters further limits mucosal damage. However, cavernous hemangiomas create a heat sink effect due to their configuration of large venous sinusoids that dissipates the laser energy and significantly reduces the efficiency of the Nd:YAG's coagulation effects. By compressing the lesion with a glass slide held with microlaryngeal forceps, the heat sink effect is mitigated and photocoagulation occurs using less total energy.[5] Thus, the risk of transmural injury to underlying structures is reduced while allowing photoablation of the hemangioma. Theoretically, since there is less damage to the surrounding normal structures, postoperative cicatrix should also be reduced.

We present three cases in which this technique was used successfully to treat adult hemangiomas involving the larynx and hypopharynx. Details of the patients' clinical course and the technique will be dis-

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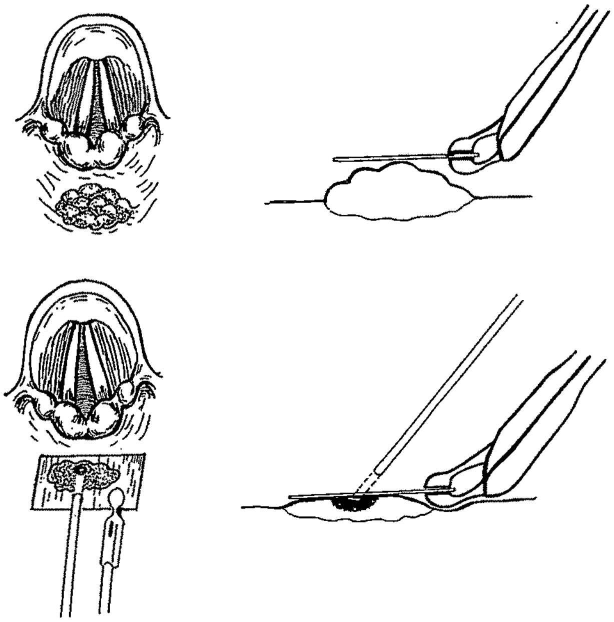

Fig 1. Glass slide held with microlaryngoscopy forceps is inserted through laryngoscope to compress laryngeal or hypopharyngeal hemangioma prior to photoablation with neodymium:yttrium-aluminum-garnet laser.

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MATERIALS AND METHODS

Three patients were treated with the commercially available SLT Contact Nd:YAG laser from SLT-YAG (Oaks, Pa) by means of the glass slide compression technique for extensive hemangiomas involving the hypopharynx and larynx over the past 3 years (1991 to 1994). A total of six treatments were performed. Follow-up after therapy has been 11 to 30 months.

The lesions were exposed with suspension microlaryngoscopy performed with general anesthesia either through a tracheostomy tube or by the jet ventilation technique. The patients' eyes were covered with moist eye pads and the face was covered with moist towels, leaving only the laryngoscope exposed. The larynx was visualized with an operative microscope and the lesion compressed with a portion of a borosilicate glass slide held by microlaryngeal forceps (Fig 1). The Nd:YAG laser was used in the continuous mode at 15 to 20 W and was transmitted through a 2.5-mm optical fiber (SLT-YAG, model SSRH 9), without a focusing tip, to the surface of the slide. The lesion was photocoagulated in a sweeping fashion until blanching and visible involution of the hemangioma were observed. With the Nd:YAG laser in the contact mode, a portion of the lesion could be resected and sent for pathologic evaluation. Each procedure took approximately 30 minutes. All patients received intraoperative and postoperative corticosteroids.

CASE REPORTS

Case 1. A 59-year-old man presented to our institution with a 2-month history of intermittent hemoptysis and a sore throat. The patient denied otalgia, a chronic cough, dysphagia, a change in voice, aspiration, shortness of breath, stridor, or weight loss. Fiber-optic and rigid endoscopic evaluation of the patient's hypopharynx and larynx demonstrated a submucosal vascular lesion extending from the right laryngeal ventricle to the posterior commissure, postcricoid region, right arytenoid, and aryepiglottic fold area. The vocal folds were normal and the airway was adequate. The remainder of the findings on examination were unremarkable. A diagnosis of a cavernous hemangioma was made.

A computed tomography (CT) scan demonstrated a vascular tumor in the right supraglottic larynx, based along the aryepiglottic fold and interposed between the endolarynx and the pyriform sinus, with extension inferiorly to the postcricoid region. This was felt to represent a cavernous hemangioma. Magnetic resonance imaging (MRI) and magnetic resonance angiography (MRA) corroborated the CT findings and showed that there was no tumor vascularity supplying the laryngeal mass from either the carotid or the vertebrobasilar circulation.

Because of the extensive nature of the lesion, a staged Nd:YAG photoablation was planned. The patient underwent a prophylactic tracheostomy using local anesthesia followed by suspension microlaryngoscopy performed under general anesthesia. The hemangioma was then photocoagulated with the Nd:YAG laser set at 15 W continuous and the lesion compressed as described. Eighty percent of the lesion was photocoagulated during this procedure. There was no bleeding. The postoperative pain was negligible and was successfully managed with acetaminophen. The patient was initially fed through a nasogastric tube and was advanced to a regular diet by the fourth postoperative day. He was discharged on the fifth postoperative day with a metallic No. 4 tracheostomy tube in place. The patient was returned to the operating room 5 weeks after his initial procedure for additional photocoagulation using the same technique to treat residual hemangioma of the postcricoid region on the right side. This was performed as an ambulatory procedure. The patient did well and was decannulated 2 weeks later.

The 1-year follow-up revealed no residual hemangioma or scar formation and a normal airway. However, the patient returned 2 years after the first procedure complaining of throat discomfort. Evaluation revealed a small hemangioma of the right arytenoid and aryepiglottic fold. No scar formation was noted

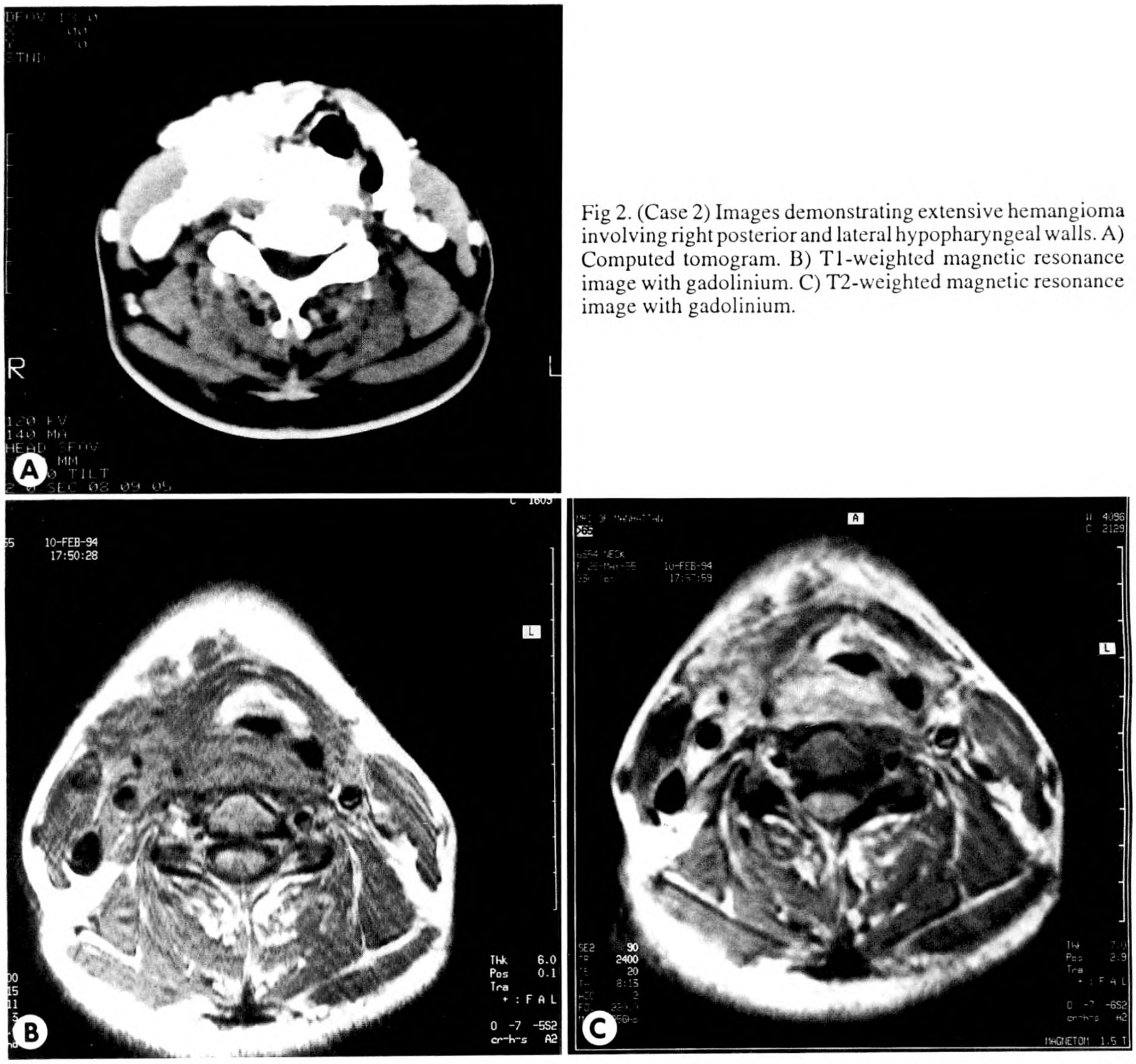

Fig 2. (Case 2) Images demonstrating extensive hemangioma involving right posterior and lateral hypopharyngeal walls. A) Computed tomogram. B) T1-weighted magnetic resonance image with gadolinium. C) T2-weighted magnetic resonance image with gadolinium.

and the vocal folds were normal. The patient was returned to the operating room and the lesion treated without a tracheostomy. Jet ventilation anesthesia was employed and the tumor photocoagulated by means of the endoscopic glass slide compression technique with the Nd:YAG laser set at 20 W. The patient was discharged the following morning.

It has been 6 months since the last procedure and the patient is doing well, without any sequelae from his Nd:YAG phototherapy and with no evidence of hemangioma.

Case 2. A 39-year-old woman presented with a painless swelling in the right side of her neck that had been gradually enlarging over the past several months. The patient admitted to a globus sensation and mild dysphagia. However, she denied odynophagia, a change in voice, respiratory difficulty, hemoptysis, weight loss, otalgia, fever, or any other medical problems. On physical examination there was a right-sided neck mass at level III that was soft, nontender, and compressible. Flexible fiberoptic nasopharyngoscopy demonstrated a large, violaceous, nonpulsatile submucosal mass in the hypopharynx. The endolarynx was normal. Examination of the oral cavity revealed this mass to involve the right side of the floor of the mouth anteriorly, extending to the area of the horizontal ramus of the mandible.

A CT scan with contrast (Fig 2A) revealed multiple, irregular foci of intense enhancement involving predominately the right anterior floor of the mouth, as well as the posterior and lateral oral and hypopharyngeal walls. These findings were consistent with a cavernous hemangioma, and MRI and MRA scans (Fig 2B,C) were performed to identify the vascular extent of the tumor. They demonstrated a predominately low-flow vascular lesion composed of large vascular spaces. Few vascular voids were noted. This was felt to be consistent with a cavernous hemangioma.

The patient underwent an angiogram that demonstrated an extensive cavernous hemangioma extending from the right floor of the mouth to the hypopharynx and involving the right thyroid lobe and a portion of the superior mediastinum. Because of the extent of the lesion, staged operative ablation was planned. The patient first underwent superselective embolization followed by an open neck exploration and excision of the hemangioma of the right thyroid lobe and superior mediastinum. The patient had a satisfactory postoperative course.

Staged Nd:YAG photocoagulation procedures were then planned to eradicate the remaining tumor. One month following the open surgical procedure, the patient underwent suspension microlaryngoscopy using jet ventilation anesthesia that revealed a cavernous hemangioma of the right hypopharynx, extending into the region of the right and left aryepiglottic folds. We performed Nd:YAG photocoagulation with the laser set at 15 W in the continuous mode and the lesion compressed with a glass slide as described. Approximately 75% of the lesion was photoablated, with a significant reduction in the size of the lesion. The patient had an excellent postoperative recovery, with no airway distress, and was discharged the following morning.

Nine months following the first laser treatment, the patient was returned to the operating room for further Nd:YAG photocoagulation therapy of the remaining hemangioma on the left aryepiglottic fold. An identical surgical technique was utilized. The patient has done well postoperatively, with full resolution of her symptoms. A 6-month follow-up examination in the office revealed no residual hemangioma or postoperative cicatrix.

Case 3. A 61-year-old woman presented complaining of discomfort in her throat and a globus sensation that had lasted for several months. She denied dysphagia, odynophagia, a change in voice, respiratory difficulty, hemoptysis, weight loss, otalgia, fever, or any other medical problems. Examination with both rigid and flexible endoscopy revealed a submucosal, nonpulsatile mass with a bluish hue involving the right arytenoid with extension to the aryepiglottic fold. The remainder of the head and neck examination findings were normal. Given the classic appearance of the tumor, the diagnosis of a laryngeal hemangioma was made.

She underwent suspension microlaryngoscopy with jet ventilation and compression photocoagulation of the lesion with the Nd:YAG laser set at 15 W continuous. Postoperatively, the patient did very well and was discharged home the following morning.

It has been 11 months since surgery and the patient has complete resolution of her preoperative symptoms. There is no evidence of residual tumor, scarring, or alteration of voice quality.

RESULTS

The patients in this series experienced total resolution of their preoperative symptoms. No untoward events occurred from the use of the laser. Excessive bleeding or airway compromise was not encountered in any of these patients. Postoperative pain was minimal at the surgical site. No mucosal sloughing was encountered. Deglutition improved in the second patient and was unimpaired in the first and third patients. On 6- to 11-month follow-up from the last procedure, the patients are without evidence of disease. No scarring or stenosis has been observed in either the hypopharynx or larynx, and the patients' phonation has been unaffected.

DISCUSSION

Cavernous hemangiomas of the larynx and hypopharynx are unusual lesions that have been a challenging clinical problem. Traditionally, treating these lesions required extensive, open surgical procedures in which patients often required numerous blood transfusions, with all of the associated morbidity and potential postoperative complications that these surgical procedures entail. Photocoagulation with the Nd:YAG laser has offered the clinician an effective alternative therapy that is minimally invasive and has few complications when used safely and correctly.

Although the Nd:YAG laser has been used to treat vascular lesions in the larynx,[5,6] large cavernous hemangiomas create a heat sink effect due to their anatomic configuration. If one tried to photocoagulate the lesion without compressing it first, much greater energy would be required to overcome the absorption of the venous sinusoids. This excess energy is transmitted well beyond the contact point of the Nd:YAG laser because of its tissue scatter characteristics. Furthermore, if one were to compress and laser-coagulate the entire lesion at one sitting, it would not decompress satisfactorily. There would be excessive extravasation of the contents of the hemangioma into adjacent normal tissue or rupture of the capsule with excessive bleeding. If we allow time between the laser procedures, scarring of the tumor bed occurs and decreases the size of the hemangioma. Therefore, treatment for large hemangiomas should be performed in stages. Since hemangiomas are benign lesions, it is better to undertreat them and follow their involution over time than to damage healthy tissue and induce excessive necrosis, edema, scarring, and stenosis, particularly when dealing with lesions in the larynx and hypopharynx.

Patients should be informed, during preoperative counseling, of this staged surgical approach. Furthermore, it should be stressed that the goal of therapy is directed at control of the tumor and relief of symptoms rather than total eradication of the lesion. With this philosophy in mind, the surgeon is encouraged to preserve normal structures and function.

There was no intraoperative or postoperative hemorrhage. Considering the vascular nature of these tumors, the Nd:YAG laser has proven to be an effective photocoagulator, even when the lesions are relatively deep and submucosal. Because of the intimate relationship of vital, named vessels to the larynx and hypopharynx, the surgeon should appreciate the proximity of these structures to the hemangioma being treated. Preoperative CT scan with contrast, MRA, and angiography are helpful in both planning the procedure and avoiding injury to adjacent vessels. Furthermore, by using tumor blanch as the treatment end point and not re-treating the same area twice, the likelihood of inadvertent damage to underlying structures is exceedingly small. As expected, the laser left the uninvolved overlying mucosa intact.

Our patients tolerated the procedures very well. There was minimal postoperative pain, requiring only acetaminophen for relief. It is felt that this can be attributed to the tissue-selective nature of the Nd:YAG laser. Because of the nerves' white color and the Nd:YAG's selective absorption by vascular tissue, the laser is able to photocoagulate effectively while sparing the nerves. Our patients had a sensate operative site following surgery, which confirmed the nerve's function postoperatively.[5] The nerves will be damaged if directly lasered. Therefore, the surgeon should have an understanding of the underlying neural anatomy and its relationship to the lesion in order to avoid iatrogenic injury. Given the benign nature of these tumors, preservation of all normal structures should be paramount.

Although Nd:YAG photoablation induces an inflammatory response, it has been shown that it causes relatively minimal tissue edema.[6] None of the patients had an edematous, compromised airway following any of the procedures. The patency of the airway was also aided by the fact that therapy was performed in stages for the larger lesions. The patients also received intraoperative and postoperative corticosteroids empirically. Our first patient had a prophylactic tracheostomy and was fed through a nasogastric tube for several days. In retrospect, both of these precautions were probably unnecessary. However, if a tracheostomy is not performed, staged procedures should be spaced several months apart to allow for full resolution of the postoperative edema and involution of the tumor.

Follow-up since the first laser procedure for each patient has been 30 months, 15 months, and 11 months for cases 1, 2, and 3, respectively. No evidence of scarring, voice change, airway compromise, or dysphagia has occurred. It is felt that the use of the glass slide compression technique significantly contributed to these results. In addition, it should be stressed that no lesion was treated repeatedly in the same site; this policy also limited the total energy delivered to the tissues and thus minimized postoperative cicatrix. Although our initial results appear encouraging, this analysis must be interpreted with caution because of the small number of patients and relatively short follow-up period.

In conclusion, the endoscopic glass slide compression–Nd:YAG laser technique has proven to be an effective tool in the treatment of laryngeal and hypopharyngeal hemangiomas. For this technique to work properly, patients should be treated in stages, particularly when the lesions are large. Therapy can be performed on an overnight basis with minimal postoperative morbidity. Excellent long-term function and full resolution of symptoms can be obtained.

ACKNOWLEDGMENT — We thank Dr Mouwafak Al-Rawi for his artistic assistance.

REFERENCES

1. Bardach J, Panje W. Surgical management of large cavernous hemangioma. Otolaryngol Head Neck Surg 1981;89:792-6.

2. Azzolini A, Bertani A, Riberti C. Superselective embolization and immediate surgical treatment: our present approach to treatment of large vascular hemangiomas of the face. Ann Plast Surg 1982;9:42-60.

3. Parkin JL, Dixon JA. Argon laser treatment of head and neck vascular lesions. Otolaryngol Head Neck Surg 1985;93:211-6.

4. Healy G, McGill T, Friedman EM. Carbon dioxide laser in subglottic hemangioma. An update. Ann Otol Rhinol Laryngol 1984;93:370-3.

5. Rebeiz E, April MM, Bohigian RK, Shapshay SM. Nd-YAG laser treatment of venous malformations of the head and neck: an update. Otolaryngol Head Neck Surg 1991;105:655-61.

6. Shapshay SM, David LM, Zeitels S. Neodymium-YAG laser photocoagulation of hemangiomas of the head and neck. Laryngoscope 1987;97:323-30.

7. Rosenfeld H, Sherman R. Treatment of cutaneous and deep vascular lesions with the Nd:YAG laser. Lasers Surg Med 1986;6:20-3.

8. Werkhaven J, Ossoff RH. Surgery for benign lesions of the glottis. Otolaryngol Clin North Am 1991;24:1179-89.

9. Mulliken JB. Classifications of vascular birthmarks. In: Mulliken JB, Young AE, eds. Vascular birthmarks: hemangiomas and malformations. Philadelphia, Pa: WB Saunders, 1988:24-37.

10. McCaffrey TV, Cortese DA. Neodymium:YAG laser treatment of subglottic hemangioma. Otolaryngol Head Neck Surg 1986;94:382-4.

SUPRAESOPHAGEAL COMPLICATIONS OF REFLUX DISEASE

Supraesophageal Complications of Reflux Disease will be held November 21-23, 1996, in Tucson, Arizona. For further information, contact Reza Shaker, MD, MCW Dysphagia Institute, Division of Gastroenterology, Froedtert Memorial Lutheran Hospital, 9200 W Wisconsin Ave, Milwaukee, WI 53226; telephone (414) 259-2742; fax (414) 384-8480.

EUROPEAN WORKING GROUP ON GENETICS OF HEARING IMPAIRMENT

The Second HEAR Project Workshop will be held in Milan, Italy, October 11-12, 1996. For further information and submission of abstracts, please contact: Alessandro Martini, MD, Professor of Audiology, Servizio di Audiologia, Clinica ORL, Università degli Studi, Corso Giovecca 203, I-44100 Ferrara, Italy; telephone: +39 532 295429 or 295341; fax: +39 532 295887; E-mail: mma@ ifeuniv.unife.it

P 24,712

Ann Otol Rhinol Laryngol 105:1996

EVALUATION AND MANAGEMENT OF HEMOPTYSIS IN INFANTS AND CHILDREN

A REPORT OF NINE CASES

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Hemoptysis is an occasional complication of adult pulmonary disease and is rare in children. The most common causes in adults are infection, bronchiectasis, pulmonary neoplasm, cystic fibrosis, pulmonary infarct, and trauma; in children the most common causes are infection and congenital abnormalities of the cardiopulmonary vasculature. Nine cases of hemoptysis in seven infants and two children will be presented. Two cases were fatal, thus illustrating the importance of rapid and definitive therapy early in the course. One of the fatal cases is the first case report in the otolaryngological literature of fatal hemoptysis in the newborn as a result of vascular anomalies associated with an absent left pulmonary artery. Eight cases of various causes, including small vessel vascular abnormality, trauma, recurrent pulmonary infection, and laryngotracheal papilloma, are discussed. The literature is reviewed, embryology is discussed, and a mechanism of death is theorized for the patient with the congenital vessel anomaly. Management considerations for hemoptysis in infants and children are discussed.

KEY WORDS — child, hemoptysis, infant, trauma, vascular anomaly.

INTRODUCTION

Hemoptysis is one of the most frightening and potentially life-threatening manifestations of cardiopulmonary disease. Prompt, aggressive, and thorough evaluation is necessary, although the search for a cause can be tedious and unrewarding. While hemoptysis may be the presenting symptom of various infectious pulmonary diseases, other causes must be considered. The most common causes of hemoptysis in adults are infection, bronchiectasis, pulmonary neoplasm, cystic fibrosis, pulmonary infarct, and trauma. In children the most common cause is infections, but congenital abnormalities of the cardiopulmonary vasculature must be considered. Two fatal and seven nonfatal cases of hemoptysis in infants and children are presented regarding the etiology, diagnostic evaluation, and management of this potentially lethal process. The fatal cases will be discussed in depth.

CASE REPORTS

Case 1 (Abnormal Pulmonary Vasculature). A 4-month-old male infant, product of an uncomplicated full-term pregnancy, presented with perioral cyanosis at birth. At 3 weeks of age, he developed respiratory distress and required oxygen and antibiotics initially. He was discharged home with the diagnosis of severe atelectasis. One month later he developed significant respiratory distress requiring intubation for presumed moderate bronchopulmonary dysplasia. On the fifth day of hospitalization he had a massive hemoptysis. An emergency laryngoscopy and bronchoscopy were performed. The child was found to have a "stovepipe"-type narrowing of the lower third of the trachea with a constant flow of blood from the left main stem bronchus. This was controlled by selective Fogarty balloon catheter tamponade for 10 minutes. The infant's condition was stabilized initially with crystalloids and then a transfusion. An arteriogram revealed that the major vessels were normal, with no bleeding site. However, the study was felt to be suboptimal because of a possible thrombosis or inadequate filling or enhancement of the left pulmonary artery. The child had a massive pulmonary hemorrhage later that evening and exsanguinated. At autopsy the findings included a hypoplastic left lung, absence of the left pulmonary artery, and a large bronchial artery arising from the descending aorta, providing the blood supply to the left lung. This vessel tunneled through the wall of the esophageal musculature and entered the left lung at its hilum.

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Presented at the meeting of the American Broncho-Esophagological Association, Palm Desert, California, May 2-4, 1995.

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SUMMARY OF CASES

Case	*Age*	*Sex*	*Signs and Symptoms*	*Studies*	*Findings at Bronchoscopy*	*Cause*	*Treatment*
3	1 y	M	Acute persistent hemoptysis	Chest radiography; clotting studies normal; purified protein derivative test negative	Superficial vessels	Unknown	Topical 1/4% phenylephrine solution
4	6 mo	M	Acute resolved hemoptysis	Chest radiography	Not performed	Trauma	Observation
5	4 mo	F	2-d history of intermittent hemoptysis, emesis, shortness of breath; no fever; white blood cell count 16.9; 33 neutrophils	Chest radiography, minimal infiltrate at base; clotting studies normal; computed tomography scan and chest normal	Diffuse tracheitis	*Chylamydia trachomatis* pneumonia	Intravenous and oral cephalosporins
6	2 y	M	3-d history of intermittent hemoptysis, cough, irritability; white blood cell count 19.7; 29 neutrophils	Chest radiography, right middle lobe pneumonia	Not performed	Pneumonia	Intravenous and oral cefuroxime sodium
7	2 mo	F	3-d history of productive cough, intermittent hemoptysis; white blood cell count 12.2; 47 neutrophils	Chest radiography, right upper lobe collapse	Pus from both main stem bronchi	*Streptococcus viridans; Enterobacter cloacae* pneumonia	Cefuroxime sodium
8	5 y	M	Persistent bleeding at endoscopy		Extensive papilloma	Laryngotracheal papilloma	Potassium titanyl phosphate laser
9	12 y	F	Persistent bleeding at endoscopy		Papilloma at carina	Laryngotracheal papilloma	Potassium titanyl phosphate laser

Also, the child had complete tracheal rings from the middle part of the trachea to the carina and a bridging bronchus whose airflow originated from a secondary bifurcation from the left main stem bronchus to the right lung. Severe abnormalities of the heart, liver, and kidneys were also found.

Case 2 (Facial Fractures). An 8-month-old female infant passenger was severely injured in a high-speed automobile accident. In the emergency room the child was noted to have gross blood coming from the mouth and nose and a rapidly enlarging abdomen that requiring emergent exploration. During intubation, blood was observed arising from the nasopharynx and subglottic region. The child became hypovolemic and suffered several cardiac arrests during the abdominal exploration. Large amounts of blood-stained froth were suctioned from the endotracheal tube by anesthesia. An intraoperative otolaryngology consultation was obtained. By means of a small flexible fiberoptic bronchoscope in the endotracheal tube, a moderate amount of nonclotting blood was visualized coming from the right main stem bronchus. Also, blood was still flowing from the infant's nose and mouth. Digital examination of the mid-face revealed multiple facial fractures. A posterior nasal pack with a Foley catheter and bilateral anterior gauze packs were placed to control the bleeding. The child suffered another hypovolemic cardiac arrest at the conclusion of the packing and could not be resuscitated.

The remaining seven cases of hemoptysis are summarized in the Table, and the various causes and their management will be discussed.

DISCUSSION

The purpose of this paper is to highlight the diagnostic evaluation and management of various causes of hemoptysis in infants and children. The evolving role of the laser will be discussed. An excellent monologue on hemoptysis in older children was written in 1980,[1] but little is written in the otolaryngology literature on hemoptysis in infants and young children.

Vascular Anomalies. Abnormalities of the tracheobronchial tree are rare, but can be devastating. A literature review found that autopsy showed an incidence of 0.1% to 2.0%, with the most common abnormality of the tracheobronchial tree being an altered takeoff of one of the segmental bronchi.[2]

Abnormalities of the pulmonary vessels are uncommon. The number of reported abnormalities in nonautopsy cases is increasing, owing to the accuracy and frequency of diagnostic imaging studies such as arteriography, magnetic resonance imaging, and computed tomography scans. Presbitero[3] reported pulmonary vascular abnormalities in 12 asymptomatic children during angiography, which were later confirmed at cardiac surgery. The typical vascular lesions include stenosis, atresia, and absence of the right pulmonary artery. Aberrant origins of the pulmonary arteries have been reported by many authors.[4] The absence of the left pulmonary artery is rare and is usually reported at autopsy. The major vessels supplying the lungs originate from the ascending aorta and occasionally from the descending aorta. The bronchial arteries will supply the lungs in the presence of pulmonary atresia or severe stenosis.

Embryologically, the pulmonary artery system arises from three loci. The main pulmonary artery arises from the truncus arteriosus, with the right and left arteries originating from the proximal sixth aortic arches; the intrapulmonary arteries arise from the primitive lung plexus. Before the major vessels develop, the lung parenchyma is supplied by small transitory branches off the dorsal aorta, which normally involute before birth. However, in cases of absent pulmonary arteries, these branches enlarge and persist as subdiaphragmatic branches, supradiaphragmatic branches, or midthoracic bronchial arteries.[4,5]

Hemoptysis is associated with tuberculosis, bronchiectasis, or pulmonary neoplasms in adults. Only 10% of adults with an absent pulmonary artery present with hemoptysis.[6] Death in these patients is theorized to be due to rupture of the thin-walled venules in the parenchyma that are subjected to the high systemic pressure directly from the aorta. Pool et al[7] found that the bronchial blood flow in these cases can be 25 times the usual rate.

Diagnosis of vascular anomalies requires a high index of suspicion. Cogswell and Singh[8] found that most adults with isolated agenesis of a pulmonary artery are asymptomatic, but that up to 40% of patients may suffer from recurrent pulmonary infections. Usually, infants and children are not tachypneic, unless they are actively bleeding. Physical findings can include an asymmetric thorax, mediastinal shift, decreased breath sounds in the affected side, and an occasional systolic murmur at the heart base due to turbulent flow into the remaining pulmonary artery. A ventilation-perfusion scan would reveal intact ventilation with altered perfusion on the affected lung. Pulmonary arteriography will confirm the absence of a pulmonary artery. Bronchoscopic findings can be normal in cases of minor hemoptysis, but bronchoscopy may be useful in localizing the site of bleeding.

With ultrasonography, the diagnosis may be made in the prenatal period. With early detection and a higher index of suspicion, an insightful otolaryngologist and a skillful interventional radiologist and thoracic surgeon can make the survival of even the smallest neonate with an absent pulmonary artery a possibility. Management of unilateral pulmonary agenesis is conservative, and hemoptysis is usually absent or minor.

In patients with massive hemoptysis, the first priority is to maintain the airway, optimize oxygenation, and stabilize the hemodynamic status. Early intubation is necessary to secure the airway. An emergent bronchoscopy during active bleeding should be performed to localize and identify the specific site of hemorrhage. Bleeding may be controlled with topical therapy, bronchoscopic pressure, or endobronchial tamponade. If bleeding continues or cannot be localized, emergent arteriography is indicated. Bronchial artery embolization is an established treatment for massive and serious hemoptysis, with good results and acceptable morbidity.[9,10]

Emergency surgical intervention for this problem should be considered when embolization has failed or is not feasible. Various techniques have been utilized to treat persistent, severe hemoptysis. Methods have included pneumonectomy in an adult, reported by Mehta et al.[2] Pneumonectomy and embolectomy were reported in a 10-year-old child by Taguchi et al.[11] A staged extra-anatomic conduit to deliver low-pressure blood to the lung, and interruption of the high-pressure vessels were reported in a 14-year-old child by Rankin et al.[12] Kirkpatrick et al[13] succeeded with a complete anatomic repair in the treatment of a symptomatic 2-month-old in high-output cardiac failure.

Our fourth case reveals that bleeding may occur spontaneously from superficially exposed vessels in the tracheobronchial tree without a predisposing infection or trauma. The diagnosis is made with either flexible or rigid bronchoscopy, and management is dependent on the severity of the hemorrhage. As with vascular anomalies, laser coagulation, embolization, or even a lobar or entire pneumonectomy may be necessary to control the bleeding. Many cases are reported without a site or cause ever being found. These cases are the most perplexing and potentially the most concerning.

Trauma. The second and third cases illustrated that pulmonary hemorrhage associated with trauma can be distracting, but must be placed in the proper perspective, during the triage of injuries. Blood coming from the airway is less worrisome, however, if air exchange is adequate; and attention should be turned to more immediately life-threatening injuries.

In children, hemoptysis can occur in posttraumatic pulmonary contusion with associated multiple trauma.[14] True tracheal or bronchial separation or rupture is rare and is usually associated with a pneumomediastinum and subcutaneous emphysema. The otolaryngologist should actively participate in the management of patients with blood in their airways and also assist the primary caregivers in establishing priorities for the treatment of other head and neck injuries.

Diagnosis of a tracheal or bronchial injury should be made either with a rigid ventilating bronchoscope with telescopic capabilities or with a flexible fiberoptic bronchoscope. The latter may be dangerous because of the inability to ventilate effectively against resistance and the difficulty in removing large clots through the small suction lumens or small endotracheal tubes used in infants.

Surgical intervention is indicated for rare cases of massive hemorrhage. The argon and potassium titanyl phosphate lasers offer hemostatic capabilities that should be considered. The application of phenylephrine hydrochloride through the ventilating bronchoscope is a last resort if lasers are not available. Unilateral life-threatening hemorrhage can be managed by tamponade with a small 1-mL Fogarty catheter, which can hold several milliliters of air.

Infectious Disease. The fifth, sixth, and seventh cases illustrate that tracheobronchial or pneumonic infection is a significant cause of hemoptysis and accounted for 29% of our cases. Infection caused by bacteria, fungi, flukes (in adults), or viruses has been reported to cause hemoptysis. The infectious process can be primarily located in the larynx, trachea, bronchi, or pulmonary parenchyma. Tuberculosis is the most common offender in adults, but *Chlamydia trachomatis* is only one of many organisms that can cause pulmonary infection in children.

Diagnosis is best made by bronchoscopy and bronchial lavage. Biopsy is usually required for *Pneumocystis carinii* infection. This can be obtained with either a flexible or rigid endoscope. Appropriate cultures from sputum, gastric aspirate, and stool are also indicated. Management involves appropriate antibiotic or antifungal medications, pulmonary toilet, humidification, and aerosol treatments.

Laryngeal and Tracheal Papillomas. The eight and ninth cases are included to demonstrate that hemorrhage from juvenile laryngeal papillomatosis can be effectively managed with the potassium titanyl phosphate laser in children. In an infant the limiting factor may be the diameter of the airway. As technology improves and miniaturization of laser fibers progresses, size may be only a temporary limitation to its use.

CONCLUSION

Hemorrhage into the infant airway is a frightening and potentially lethal event. The differential diagnosis in children includes cardiopulmonary vascular malformations, bronchiectasis, cystic fibrosis, trauma, papilloma, polyps, vasculitis, idiopathic pulmonary hemosiderosis, and infection. The most perplexing and disconcerting cases are the idiopathic ones. The fatal consequences of misdiagnosis in vascular and traumatic lesions have been demonstrated by the two illustrated cases. It is hoped that these case reports will heighten the index of suspicion of physicians and increase survival in these patients.

REFERENCES

1. Tom LWC, Weisman RA, Handler SD. Hemoptysis in children. Ann Otol Rhinol Laryngol 1980;89:419-24.

2. Mehta AC, Livingston DR, Kawalek W, Golish JA, O'Donnell JK. Pulmonary artery agenesis presenting as massive hemoptysis. A case report. Angiology 1987;Jan:67-70.

3. Presbitero P, Bull C, Haworth SG, de Leval MR. Absent or occult pulmonary artery. Br Heart J 1984;52:178-85.

4. Ellis K, Seaman WB, Griffiths SP. Congenital anomalies of the pulmonary arteries. Thorax 1973;28:435-7.

5. Boyden EA. The developing bronchial arteries. Am J Anat 1970;129:357.

6. Ohta T. Congenital absence of the right pulmonary artery. A case report and review. Tokai J Exp Clin Med 1983;8:97-104.

7. Pool P, Vogel J, Blount SG. Congenital unilateral absence of a pulmonary artery. Am J Cardiol 1962;10:706-32.

8. Cogswell TE, Singh S. Agenesis of the left pulmonary artery as a cause of hemoptysis. J Vasc Dis 1986;March:154-9.

9. Lampmann LE, Tjan TG. Embolization therapy in hemoptysis. Eur J Radiol 1994;18:15-9.

10. Hansen ME, Kadir S. Elective and emergency embolization therapy in children and adolescents. Efficacy and safety. Radiologe 1990;30:331-6.

11. Taguchi T, Ikeda K, Kume K, et al. Isolated unilateral absence of left pulmonary artery with peribronchial arteriovenous malformation showing recurrent hemoptysis. Pediatr Radiol 1987;17:316-8.

12. Rankin JS, Sterba RJ, Karis JH, Oldham HN Jr. Staged correction of pulmonary atresia and absent right pulmonary

artery. Arch Surg 1983;118:974-8.

13. Kirkpatrick SE, Girod DA, King H. Aortic origin of the right pulmonary artery. Surgical repair without a graft. Circulation 1967;36:777-82.

14. Bonadio WA, Hellmich T. Post-traumatic pulmonary contusion in children. Ann Emerg Med 1989;18:1050-2.

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PEDIATRIC OTORHINOLARYNGOLOGY ENDOSCOPY DAYS

The First International Days in Pediatric Otorhinolaryngology Endoscopy will be held in Lyon, France, October 14-15, 1996. For further information, contact Marie-Claude Duchez, Departement d'ORL et de Chirurgie Cervico-Faciale, Hôpital E. Herriot, Place d'Arsonval, 69003 Lyon, France; fax 33 72 11 05 34.

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INNER EAR MEDICINE AND SURGERY

The Sixth Symposium and Workshops on Inner Ear Medicine and Surgery, combined with the Prosper Meniere Society Annual Winter Meeting, will be held in Aspen/Snowmass, Colorado, March 22-29, 1997. For further information, contact Prosper Meniere Society, c/o Jane Wells, Meeting Coordinator, 300 E Hampden Ave, Suite 401, Englewood, CO 80110; telephone (303) 788-4235; fax (303) 788-4234.

Ann Otol Rhinol Laryngol 105:1996

REPEATEDLY SUCCESSFUL CLOSURE OF THE LARYNX FOR THE TREATMENT OF CHRONIC ASPIRATION WITH THE USE OF BOTULINUM TOXIN A

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Botulinum toxin A was used preoperatively to temporarily paralyze the intrinsic laryngeal muscles to hinder movements during the healing period after operation. In addition, toxin was injected into the cricopharyngeal muscle to allow a better passive drainage of the saliva into the esophagus. We treated six patients. Three suffered from chronic aspiration problems after multiple lower cranial nerve lesions, and three patients were apallic (after stroke and major brain injury). Two weeks before scheduled operation, we injected the toxin into the posterior cricoarytenoid muscles, the aryepiglottic muscles, and the vocalis muscle on both sides, as well as the cricopharyngeal muscle. The amount of injected toxin varied between 1.0 and 1.4 mL, equal to 200 to 280 units of botulinum toxin A (Dysport). After a complete palsy of these muscles (controlled by direct electromyography), a closure of the larynx was performed. After laminotomy and exposure of the intralaryngeal structures, the false vocal cords were mobilized and adapted with sutures. Because involuntary movements of the intralaryngeal musculature were absent, primary healing without complications occurred in all cases. Aspiration and related complications disappeared in all patients. In addition, the intensity of patient care could be considerably reduced. Preoperative use of botulinum toxin A allows sufficient laryngeal closure. This procedure is especially useful in the treatment of children and young adults, preserving the ability of later speech rehabilitation because of the return of voluntary movements of the intrinsic laryngeal muscles 6 months after the injection. Furthermore, this technique, as minimal surgical intervention, can be performed in high-risk patients.

KEY WORDS — botulinum toxin A, chronic aspiration, dysphagia, electromyography, laryngectomy, operative treatment.

INTRODUCTION

Sphincter insufficiency of the larynx, often caused by neurologic disorders, leads to continuous aspiration with life-threatening consequences. In the treatment of aspiration a variety of surgical techniques and conservative physiotherapy have been employed with various degrees of success.[1-6]

The goal of successful surgical treatment is the separation of the airway and the digestive tract. The most effective treatment is laryngectomy, with irreversible consequences. Therefore, surgical techniques that separate the tracheobronchial system from the digestive tract and simultaneously preserve the larynx are preferable.[7-11]

Tracheoesophageal division and laryngotracheal separation with a temporary opening of the hypopharynx and esophagus lead to numerous complications such as salivary fistula and wound healing problems.[12] The success rate of such techniques as epiglottopexy and closure of the larynx depends on the extent of the palsy of the swallowing muscles: in patients with bilateral palsy, closure of the larynx is usually successful, but in patients with central swallowing disorders and discoordinated function of the caudocranial nerves with active aspiration, this technique often fails because of strong voluntary movements.[13-16] Ineffective and repeated swallowing trials with strong movements in the glottic area cause suture insufficiency with recurrent aspiration. In addition, closure at the level of the glottis leads to the development of scar tissue at the vocal cords.[17-19]

It was previously shown that botulinum toxin A is a very potent drug for treating various dyskinesias in otorhinolaryngology.[20-22] The injection of botulinum toxin A causes a reversible palsy of the treated muscle by strong and rapid binding to presynaptic cholinergic nerve terminals and inhibition of the release of acetylcholine (chemical denervation). The clinical effect of botulinum toxin is temporary, lasting up to 6 months, and due primarily to its action at the neuromuscular junction.

Therefore, we used this paralyzing effect to hinder and suppress spontaneous swallowing movements during the postoperative period of larynx closure and to facilitate wound healing.

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SUMMARY OF PATIENT DATA

Pt	*Age*	*Sex*	*Basic Disease*	*Apallic*	*Aspiration Pneumonia*	*Tracheostomy*	*Nursing Intensity*
1	57	F	Endocranial tumor operation, multiple cranial nerve lesions	No	Severe	Yes	Medium
2	76	M	Severe stroke	No	Severe	Yes	High
3	42	M	Severe stroke	Yes	Severe	Yes	
4	32	F	Accident, brain injury	Yes	Severe, multiple	Yes	High
5	10	M	Accident, asphyxia	Yes	Severe, multiple	Yes	Extremely high
6	18	M	War victim (Bosnia), brain injury	No	Severe, multiple	Yes	Extremely high

METHODS

Patients. There was a total of six patients, four male and two female, between 10 and 76 years of age. All patients suffered from severe chronic aspiration caused by stroke, severe head injury, cranial tumor resection, or hypoxic trauma after drowning, leading to multiple dysfunctions and palsies with complex dyscoordination and aspiration during swallowing. Three patients were apallic.

Patient care was very demanding, and all patients had recurrent aspiration pneumonia. For example, we treated a 10-year-old boy with extensive hypersalivation, which made almost continuous suction of the saliva in the trachea necessary. All non-apallic patients were unable to talk because of multiple cranial nerve lesions.

Despite extensive physical therapy to improve swallowing, none of the patients showed persistent improvement of swallowing within up to 3 years. Therefore, the indication for surgical treatment was determined.

Laryngectomy was refused by two patients and could not be performed in one patient because of his critical situation (see Table).

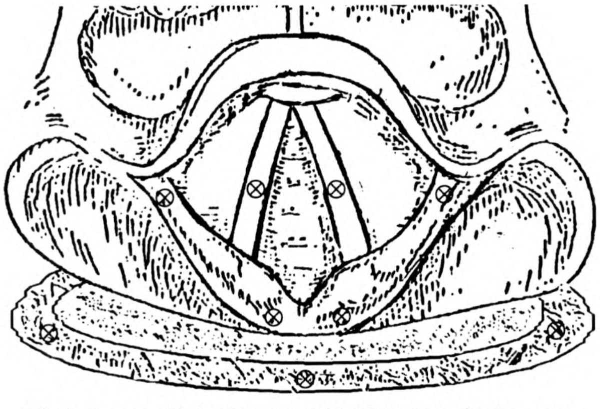


Fig 1. Areas of injection (⊗) with 20 to 30 units Dysport (Speywood), showing vocal cords, posterior cricoarytenoid muscle, lateral thyroarytenoid muscle, and cricopharyngeal muscle.

Technique. As an initial intervention, an epithelialized tracheostomy was performed to prevent further aspiration of the saliva by blocking the tube or placement of a surrounded cannula[18] or a laryngeal obturator.[23] All of these techniques are only of temporary help because of the high risk of infection and damage to the trachea in the area surrounding the block.

In order to suppress any contractions of the laryngeal musculature, botulinum toxin A was injected during microlaryngoscopy under general anesthesia 2 weeks before the scheduled closure of the larynx. Botulinum toxin A was obtained from the Speywood Company (Dysport). The lyophilized drug was diluted with 2.5 mL 0.9% sodium chloride, equivalent to 20 units per 0.1 mL.

Botulinum toxin A was injected bilaterally into the following muscles: posterior cricoarytenoid, aryepiglottic, medial thyroarytenoid (vocalis), and lateral thyroarytenoid. To accomplish complete palsy of the larynx musculature, each muscle was injected with

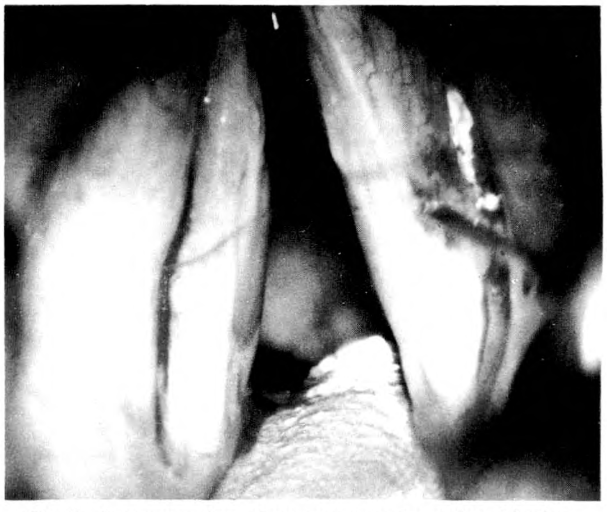


Fig 2. Intraoperative electromyography using bipolar hooked-wire electrodes.

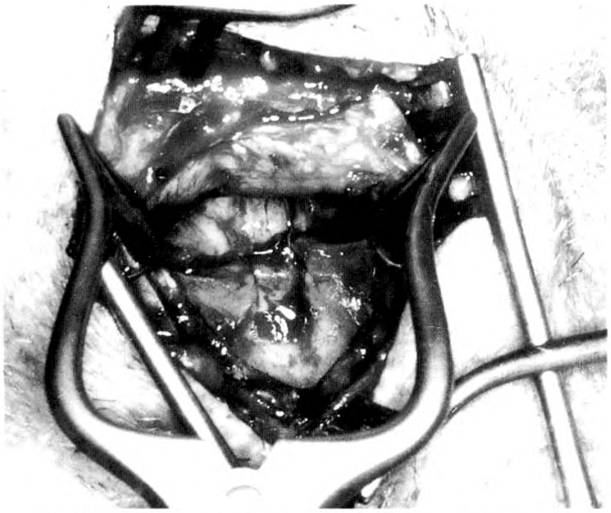


Fig 3. Intraoperative site exposing vocal cords and false vocal cords, with posterior-anterior suture.

20 to 30 units of botulinum toxin A (Fig 1).

We have previously shown[22] that the injection of botulinum toxin A into the upper esophageal sphincter prevents aspiration in some cases. The denervation of the cricopharyngeal muscle facilitates passive flow of saliva and the food bolus into the esophagus. Therefore, all patients received botulinum toxin A injected into the cricopharyngeal muscle at three sites. The total dose for complete palsy was 80 to 100 units.

On the day of operation, the completeness of the palsy was checked by intraoperative electromyography (Fig 2).[24,25] After documentation of spontaneous activity in the treated muscles, a closure of the larynx as described by Denecke[17] was performed with the following modifications: after laminotomy and exposure of the intralaryngeal structures, the false vocal cords were mobilized and adapted with sutures (Figs 3 and 4).

RESULTS

All of our treated patients showed excellent wound healing. Five of six patients had a sufficient, complete closure of the larynx. Only the 76-year-old patient suffered a very thin fistula of the posterior commissure, detected by a swallowing test with methylene blue solution.

In all patients, aspiration was completely inhibited, and the nursing intensity could be reduced. In the 10-year-old boy, hypersalivation occurred 6 months after operation, despite the use of anticholinergic agents. This could be explained by the diminishing effect of botulinum toxin A in the cricopharyngeal muscle, causing an accumulation of saliva; a reinjection of this area is planned.

A reopening of the larynx could not be performed in any of our patients, because the underlying disorders had not changed considerably.

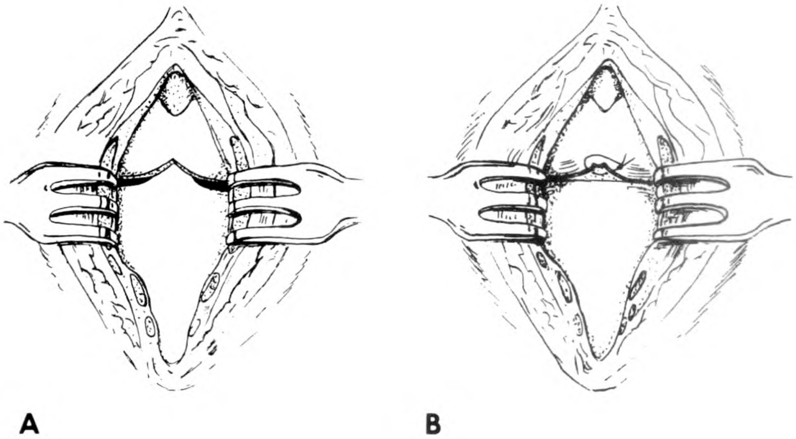
The 32-year-old woman died of multiple organ failure 1 year after laryngeal closure.

CONCLUSION

Preoperative localized injections of botulinum toxin A into the intrinsic laryngeal musculature leads to a complete paralysis of this musculature and therefore hinders voluntary movements during the healing period after operation. By this method the rate of sufficient laryngeal closure could be increased enormously. Concomitant injection into the upper esophageal sphincter allows a better passive drainage of saliva into the esophagus, and additionally prevents high pressure to the larynx.

This procedure is especially useful for the treatment of children and young adults, preserving the ability for later speech rehabilitation because of the reversibility of the paralyzing effect of botulinum toxin A on the laryngeal musculature and the gentle operative technique. Furthermore, this technique, as minimal surgical intervention, can also be performed in high-risk patients. The time for patient care could be considerably reduced.

Fig 4. Sketches of intraoperative site. A) Incision in sinus of Morgagni. B) Posterior-anterior suture of false vocal cords.



REFERENCES

1. Kitahara S, Ikeda M, Ohmae Y, Nakanoboh M, Inouye T, Healy GB. Laryngeal closure at the level of the false cord for the treatment of aspiration. J Laryngol Otol 1993;107:826-8.

2. Saltzman LS, Rosenberg CH, Wolf RH. Brainstem infarct with pharyngeal dysmotility and paralyzed vocal cord: management with a multidisciplinary approach. Arch Phys Med Rehabil 1993;74:214-6.

3. Fujita H, Hirano M, Kakegawa T, Yamana H, Tanaka S. Surgical treatment for aspiration following esophageal reconstruction — a report of two cases and the techniques involved. Jpn J Surg 1991;21:687-92.

4. Eisele DW. Surgical approaches to aspiration. Dysphagia 1991;6:71-8.

5. Blitzer A. Approaches to the patient with aspiration and swallowing disabilities. Dysphagia 1990;5:129-37.

6. Lulenski GC. Laryngeal closure and glottic reconstruction. Ear Nose Throat J 1980;59:495-8.

7. McIlwain JC, Bryce DP, Gilbert R, Grace A. Subglottic laryngeal closure for aspiration. Clin Otolaryngol 1991;16:33-8.

8. Butcher RB. Treatment of chronic aspiration as a complication of cerebrovascular accident. Laryngoscope 1982;92:681-5.

9. Nahum AM, Harris JP, Davidson TM. The patient who aspirates — diagnosis and management. J Otolaryngol 1981; 10:10-6.

10. Addy DP, Robin PE. Laryngeal closure in the management of severe long-standing bulbar palsy in a child. Int J Pediatr Otorhinolaryngol 1981;3:263-7.

11. Glenn WW, Haak B, Sasaki C, Kirchner J. Characteristics and surgical management of respiratory complications accompanying pathologic lesions of the brain stem. Ann Surg 1980;191: 655-63.

12. Lindeman RC. Diverting the paralyzed larynx: a reversible procedure for intractable aspiration. Laryngoscope 1975;85: 157-80.

13. Tucker HM. Management of the patient with an incompetent larynx. Am J Otolaryngol 1979;1:47-56.

14. Montgomery WW. Surgical laryngeal closure to eliminate chronic aspiration. N Engl J Med 1975;292:1390-1.

15. Montgomery WW. Surgery to prevent aspiration. Arch Otolaryngol 1975;101:679-82.

16. Habal MB, Murray JE. Surgical treatment of life-endangering chronic aspiration pneumonia. Use of an epiglottic flap to the arytenoids. Plast Reconstr Surg 1972;49:305-11.

17. Denecke HJ. Plastische Korrektur des Schluckaktes und der Stimme bei Vagusläsion. 19 Jahre Erfahrung. HNO 1977;25: 140-3.

18. Denecke HJ. Trachealplastik im Bereich der Halsbasis bei besonderen pathologischen Verhältnissen der oberen Thoraxapertur. HNO 1975;23:126-8.

19. Denecke HJ. Korrektur des Schluckaktes bei einseitiger Pharynx- und Larynxlähmung. HNO 1961;9:351-3.

20. Jankovic J, Brin MF. Therapeutic uses of botulinum toxin. N Engl J Med 1991;324:1186-94.

21. Blitzer A, Brin MF. Laryngeal dystonia: a series with botulinum toxin therapy. Ann Otol Rhinol Laryngol 1991;100:85-9.

22. Schneider I, Thumfart WF, Pototschnig C, Eckel HE. Treatment of dysfunction of the cricopharyngeal muscle with botulinum A toxin: introduction of a new, noninvasive method. Ann Otol Rhinol Laryngol 1994;103:31-5.

23. Weisberger EC. Treatment of intractable aspiration using a laryngeal stent or obturator. Ann Otol Rhinol Laryngol 1991; 100:101-7.

24. Thumfart WF, Pototschnig C, Gubitz J, Schneider I. Functional and electrophysiologic diagnosis of dysphagia. Trans Am Laryngol Assoc 1992;113:176-84.

25. Thumfart WF. Electromyography of the larynx and related techniques. Acta Otorhinolaryngol Belg 1986;40:358-76.

LANGUAGE DEVELOPMENT IN COCHLEAR IMPLANTED CHILDREN

The International Conference on Language Development in Cochlear Implanted Children will be held December 8-9, 1996, in Lyon, France. For more information, contact Prof A. H. Morgon, Hopital Edouard Herriot, pavillon U, place d'Arsonval, 69003 Lyon, France; telephone (33) 72.11.05.35; fax (33) 72.11.05.34.

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IMMUNOHISTOCHEMICAL CHARACTERIZATION OF BENIGN LARYNGEAL LESIONS

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It has been proposed that laryngeal nodules and polyps represent injury to the basement membrane zone of the vocal fold. Repeated trauma from shearing forces produced by excessive or abusive phonation leads to basement membrane zone disruption and thickening. This thickening, along with poorly understood vascular changes, creates the characteristic clinical appearance of the vocal nodule or polyp. As such, to better understand vocal fold nodules it is imperative to characterize the extracellular matrix in this area of injury. Secondary to the small size and relatively acellular nature of these lesions, hematoxylin and eosin (H & E) preparations of histologic material are unsatisfying. A previous study examined this area with immunohistochemical techniques to better characterize its contents. The report, however, contained little information with regard to the clinical appearance of the lesions prior to excision. Therefore, we were prompted to review histologic material from 31 patients who underwent microsurgical excision of 41 benign lesions, vocal nodules (4), polyps (19), polypoid corditis (4), and cysts (14) with immunohistochemical techniques to characterize the patterns of fibronectin and collagen type IV within these lesions. Normal human vocal folds were stained for control. All material was correlated with the H & E preparations and the clinical diagnosis. Collagen type IV and fibronectin appeared present in relatively abnormal patterns in the areas adjacent to the lesion. This study validates earlier results. In addition, correlation with clinical data allows association of immunohistochemical staining patterns with clinical diagnosis.

KEY WORDS — basement membrane zone, benign laryngeal lesions, collagen, cyst, fibronectin, immunohistochemistry, nodule, polyp, vocal fold.

INTRODUCTION

Through the work of Hirano and Kakita[1] and others, a greater understanding of the ultrastructure of the lamina propria and the basement membrane zone (BMZ) of the human vocal fold has been attained. This area is composed of sparse cellular material interposed with an extracellular matrix. This extracellular matrix is presumed responsible for the vibratory characteristics of the vocal fold. As such, changes in the extracellular matrix secondary to injury could have an important effect on vocal fold vibratory characteristics.

Because the lamina propria of the vocal fold is relatively acellular, study by means of hematoxylin and eosin (H & E) analysis has yielded limited information. Therefore, it has been necessary to examine this tissue by other means such as electron microscopy and immunohistochemical analysis.[2] Likewise, non-neoplastic lesions of the human vocal fold, which involve the extracellular matrix almost exclusively, are poorly understood on a histologic basis. These lesions, which represent a response of the human vocal fold, more specifically the lamina propria and BMZ, to trauma,[3] are relatively acellular. Analysis with H & E provides minimal information regarding the pathologic process. It is hoped that a more accurate analysis of the histopathology of these non-neoplastic diseases will provide a better understanding of the disease process and therefore improve future treatment methods.

In a recent study by Gray et al,[4] the pathologic response of the BMZ and the lamina propria to injury was evaluated by immunohistochemical methods. These authors studied archival material removed from human vocal folds. Because the subject material was archival, the authors were not able to correlate their findings precisely with the clinical diagnosis. Therefore, they were limited in their ability to make conclusions comparing the histologic responses to the clinical diagnosis. The present study was undertaken to verify their findings and to correlate known clinical diagnosis with histologic findings.

METHODS

Surgical specimens were obtained from Vanderbilt University Medical Center Department of Pathology. All specimens had been surgically excised by two of the authors (R.H.O. and M.S.C.) between

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Presented at the meeting of the American Broncho-Esophagological Association, Palm Desert, California, May 2-4, 1995.

REPRINTS — Mark S. Courey, MD, Dept of Otolaryngology, Vanderbilt University Medical Center, S-2100 Medical Center North, Nashville, TN 37232-2559.

SPECIMEN DATA

Specimen No.	*Patient No.*	*Clinical Diagnosis*	*Basement Membrane Width (μm)*		*Decreased Collagen IV*	*Laminar Fibronectin*	*Vascular Cuffing*
			Collagen IV	*Fibronectin*			
1	1	Nodule	2.0	2.0	No	Yes	No
2	2	Nodule	0.5	1.0	No	Yes	No
3	2	Nodule	2.0	2.0	No	Yes	No
4	3	Nodule	3.0	1.5	No	No	No
5	4	Polyp	0.5	0.5	Yes	No	No
6	5	Polyp	0.5	1.0	Yes	Yes	Yes
7	5	Polyp	1.0	1.5	Yes	Yes	Yes
8	6	Polyp	0.5	0.5	Yes	Yes	Yes
9	7	Polyp	1.0	1.0	Yes	Yes	Yes
10	8	Polyp	1.0	1.0	Yes	No	Yes
11	9	Polyp	0.5	1.0	Yes	Yes	Yes
12	10	Polyp	1.0	1.0	Yes	Yes	Yes
13	11	Polyp	0.5	1.0	Yes	Yes	No
14	12	Polyp	1.0	1.0	No	Yes	Yes
15	13	Polyp	1.5	2.0	No	Yes	Yes
16	14	Polyp	0.5	1.0	Yes	Yes	No
17	15	Polyp	2.0	5.0	No	No	No
18	16	Polyp	0.5	0.5	Yes	No	Yes
19	17	Polyp	0.5	0.5	Yes	Yes	No
20	18	Polyp	0.5	0.5	Yes	Yes	Yes
21	19	Polyp	0.5	0.5	Yes	Yes	Yes
22	20	Polyp	0.5	1.0	Yes	Yes	No
23	20	Polyp	2.0	2.5	Yes	Yes	Yes
24	20	Squamous cyst	0.5	0.5	Yes	Yes	No
25	20	Squamous cyst	0.5	0.5	Yes	Yes	Yes
26	21	Squamous cyst	0.5	1.0	Yes	Yes	No
27	21	Squamous cyst	0.5	0.5	Yes	Yes	No
28	22	Mucous cyst	0.5	0.5	No	Yes	No
29	23	Squamous cyst	5.0	5.0	No	No	No
30	24	Squamous cyst	1.0	1.5	No	Yes	No
31	24	Squamous cyst	1.0	1.0	No	Yes	Yes
32	25	Mucous cyst	1.0	1.0	Yes	No	No
33	26	Mucous cyst	0.5	0.5	No	Yes	No
34	27	Mucous cyst	2.0	2.5	Yes	No	No
35	28	Mucous cyst	0.5	0.5	Yes	No	No
36	27	Mucous cyst	0.5	0.5	No	Yes	No
37	28	Mucous cyst	0.5	0.5	Yes	Yes	Yes
38	29	Polypoid corditis	1.0	1.0	No	Yes	Yes
39	29	Polypoid corditis	0.5	0.5	Yes	No	No
40	30	Polypoid corditis	1.0	1.0	Yes	Yes	Yes
41	31	Polypoid corditis	0.5	0.5	No	No	No

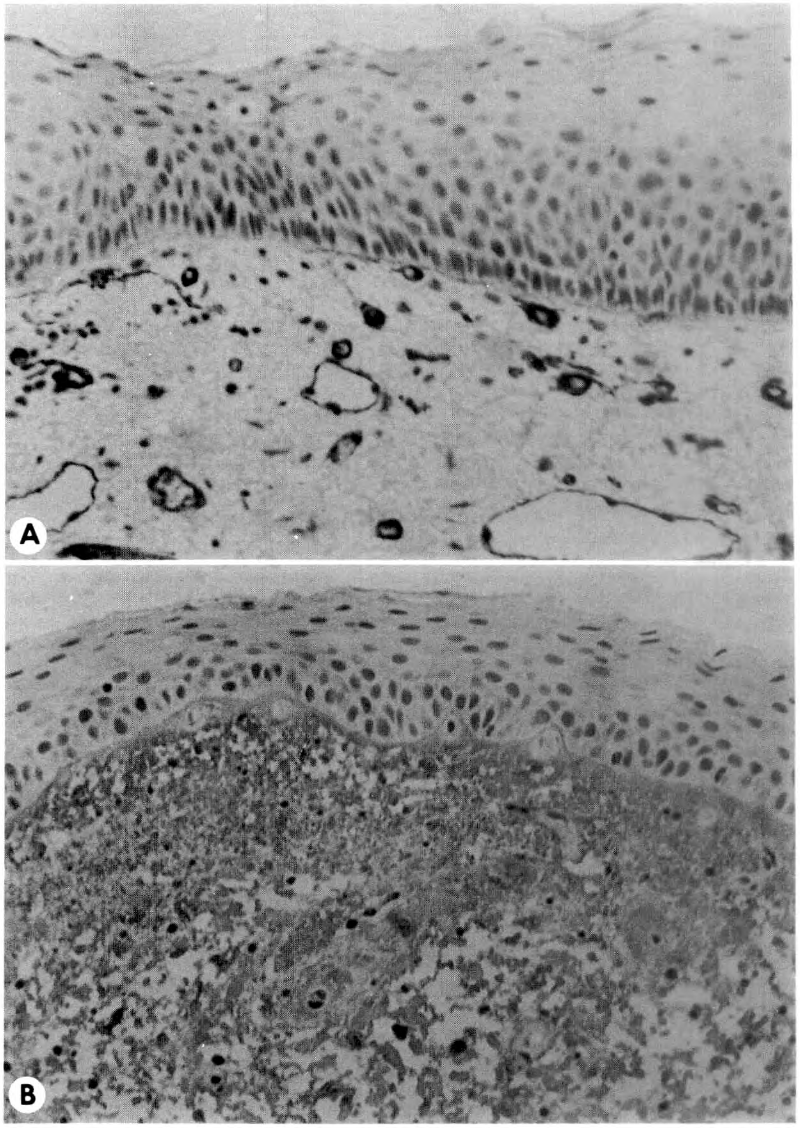

Fig 1. Control specimens (original ×25). A) On collagen type IV staining, note thin marking of basement membrane zone (BMZ) of surface epithelium. This is represented by thin, dark line immediately deep to basal layer of cells of epithelium. B) Fibronectin staining exhibits laminar pattern with more intense staining near BMZ and then reduction of staining through deeper portion of lamina propria. Fibronectin uptake is represented in this staining technique by fine fibrillar marking pattern. In these photographs fibronectin appears as shades of gray.

1991 and 1994. At the time of excision, specimens were formalin-fixed, paraffin-embedded, cut into 4-μm-thick sections, and mounted on glass slides. Previous H & E analyses were reviewed. Only specimens that contained an intact epithelial surface, BMZ, and superficial portion of the lamina propria were subjected to further analysis. The clinical diagnosis was obtained by reviewing the preoperative stroboscopic findings, clinical notes, preoperative photographs, and intraoperative findings dictated by the surgeon at the time of excision.

Specimens with the diagnosis of nodule, polyp, polypoid corditis, and cyst were included for analysis. Lesions were diagnosed as nodules only if they were bilateral, were located at the middle third of the membranous vocal fold, and did not respond to a 3-month vocal abuse reduction program. Polyps were either sessile or pedunculated and clinically had either an angiomatous or myxoid appearance. Lesions were diagnosed as polypoid corditis only if they involved the entire membranous vocal fold. Finally, lesions were diagnosed as cysts if preoperative stroboscopic examination revealed a reduced or absent mucosal wave and the excised lesion revealed a true epithelial lining on histologic examination.

Immunohistochemical analysis was undertaken with monoclonal antibodies directed against fibronectin and collagen type IV (Dako Corp, Carpinteria, Calif). A peroxidase-antiperoxidase technique was used. Analysis was performed in three separate runs on a BioTek Techmare 1000. Protease digestion was not required. Primary antibody dilutions of 1 to 50 and 1 to 8,000 were employed for collagen type IV and fibronectin, respectively.

Control specimens were obtained from autopsy material from 4 normal human larynges. The age range was from 12 to 65. The control subjects had died of unrelated causes and without a history of

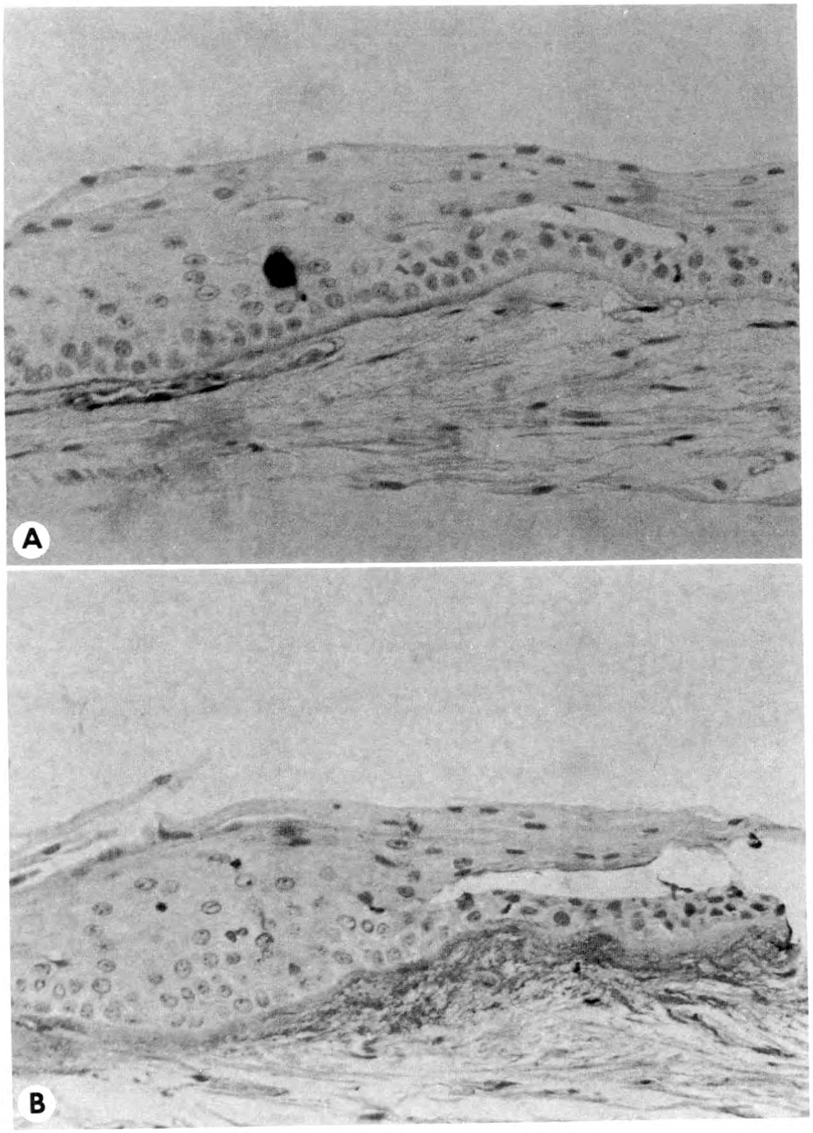

Fig 2. Clinical nodules (original ×25). A) Collagen type IV staining demonstrates thickening in BMZ, which is represented by broader marking of collagen type IV. B) Same specimen. Fibronectin staining is also more intense in immediate BMZ zone.

prolonged intubation. These were processed in the same manner as our subject material and stained simultaneously with each separate run. In addition, the normal portion of a vocal fold from a patient undergoing laryngectomy for carcinoma was used as a control.

RESULTS

Forty-one separate specimens from 31 patients provided sufficient material for analysis. These included 4 clinically diagnosed nodules, 19 polyps, 4 specimens with polypoid corditis, and 14 specimens with true vocal fold cysts (see Table).

The laryngeal control specimens all had a BMZ width of 0.5 μm or less on both collagen type IV and fibronectin staining. Collagen type IV uptake was similar in the BMZ of the covering epithelium and in the BMZ of the vasculature. Fibronectin staining was more intense in the immediate superficial lamina propria and then became less intense through the deeper layers of the lamina propria in a graded, or laminar, fashion (Fig 1).

Specimens with the clinical diagnosis of nodule showed an average BMZ thickness of 1.88 μm (range 0.5 to 3.0 μm) on collagen type IV staining and 1.63 μm (range 0.5 to 2.0 μm) on fibronectin staining. All epithelial BMZs marked intensely. When compared to the control specimen, fibronectin staining patterns in these specimens were also more intense. In 1 of the 4 nodule specimens, fibronectin had lost its normal laminar pattern. In addition, 2 of the 4 specimens had a particularly dense pattern of fibronectin deposition. There was no increased vascularity in these 4 lesions (Fig 2).

Specimens with the clinical diagnosis of polyp had an average BMZ thickness of 0.84 μm (range 0.5 to 2.0 μm) on collagen type IV staining and 1.21 μm (range 0.5 to 5.0 μm) on fibronectin staining. With 3

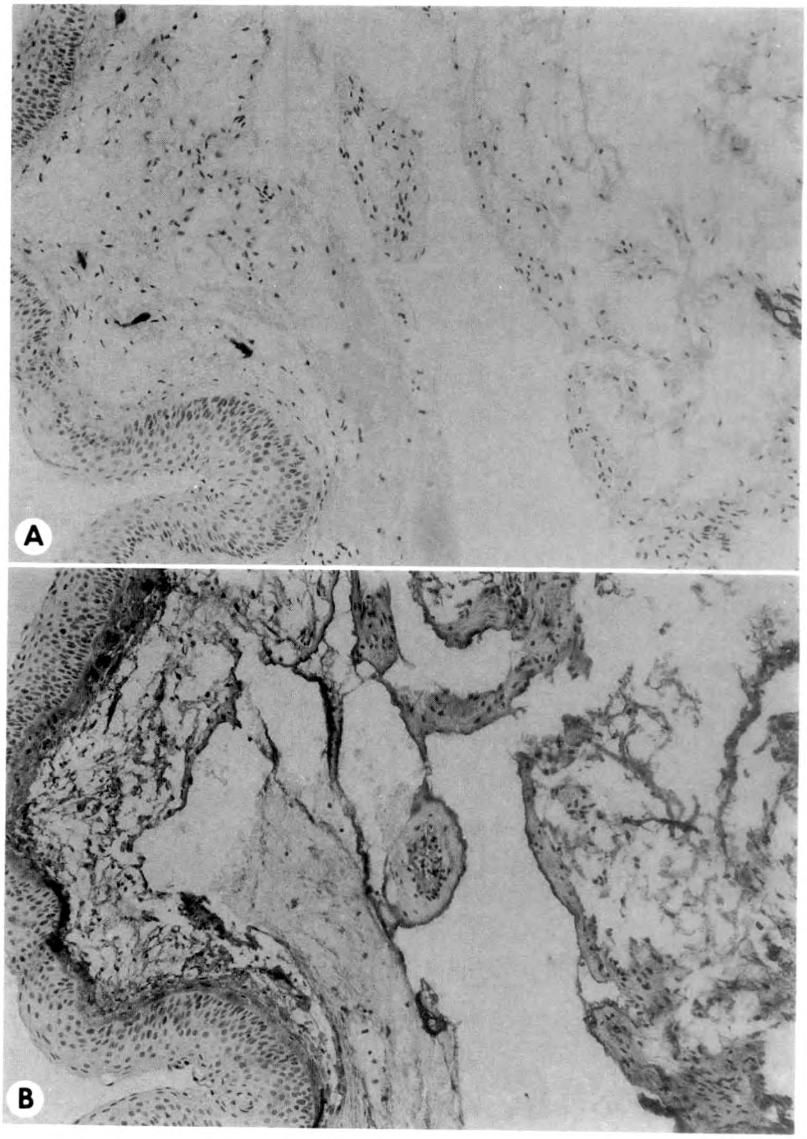

Fig 3. Clinical polyps (original ×4). A) Collagen type IV staining demonstrates decreased marking of BMZ. Collagen type IV marking is actually absent in portions of lining epithelium. B) Same specimen. Fibronectin staining has lost its laminar distribution. It is clustered in nodular pattern around neovascularity.

exceptions, collagen type IV staining demonstrated a BMZ width of 1 μm or less. The mode for this group was 0.5 μm (11 of 19). In 16 of these 19 specimens, the BMZ lining the surface epithelium seemed to take up antibodies to collagen type IV less intensely than the BMZ lining the vasculature. With regard to fibronectin uptake, in 15 of the 19 specimens, fibronectin maintained its general laminar distribution throughout the lamina propria. However, large clusters of angiomatous-appearing vessels frequently (13 of the 19 specimens) interrupted this laminar pattern, giving the impression of increased fibronectin uptake (Fig 3).

Fourteen vocal folds contained clinically diagnosed cysts of either the mucous retention type (n = 7) or the squamous inclusion type (n = 7). The characteristics regarding the immunohistochemical analysis were similar; therefore, they will be discussed as one group. The average BMZ thickness of the cyst lining was 1.04 μm on collagen type IV staining and 1.14 μm on fibronectin staining. With 2 exceptions, 1 inclusion and 1 mucous retention cyst, collagen type IV staining demonstrated a BMZ width of 1.0 μm or less. The mode for this group was 0.5 μm (9 of 14). In 8 of these 14 specimens, the BMZ lining the cyst epithelium stained less intensely for collagen type IV than did the BMZ surrounding the vasculature. With regard to fibronectin uptake, in 10 of the 14 specimens fibronectin maintained a general laminar distribution throughout the surrounding lamina propria. Only 3 specimens had associated increased vasculature that interrupted the normal laminar distribution of fibronectin (Fig 4).

Four specimens were clinically diagnosed as polypoid corditis. Collagen type IV and fibronectin staining revealed an average BMZ width of 0.75 μm. Two had a BMZ width of 0.5 μm and 2 had a BMZ width of 1.0 μm. Collagen type IV uptake was re-

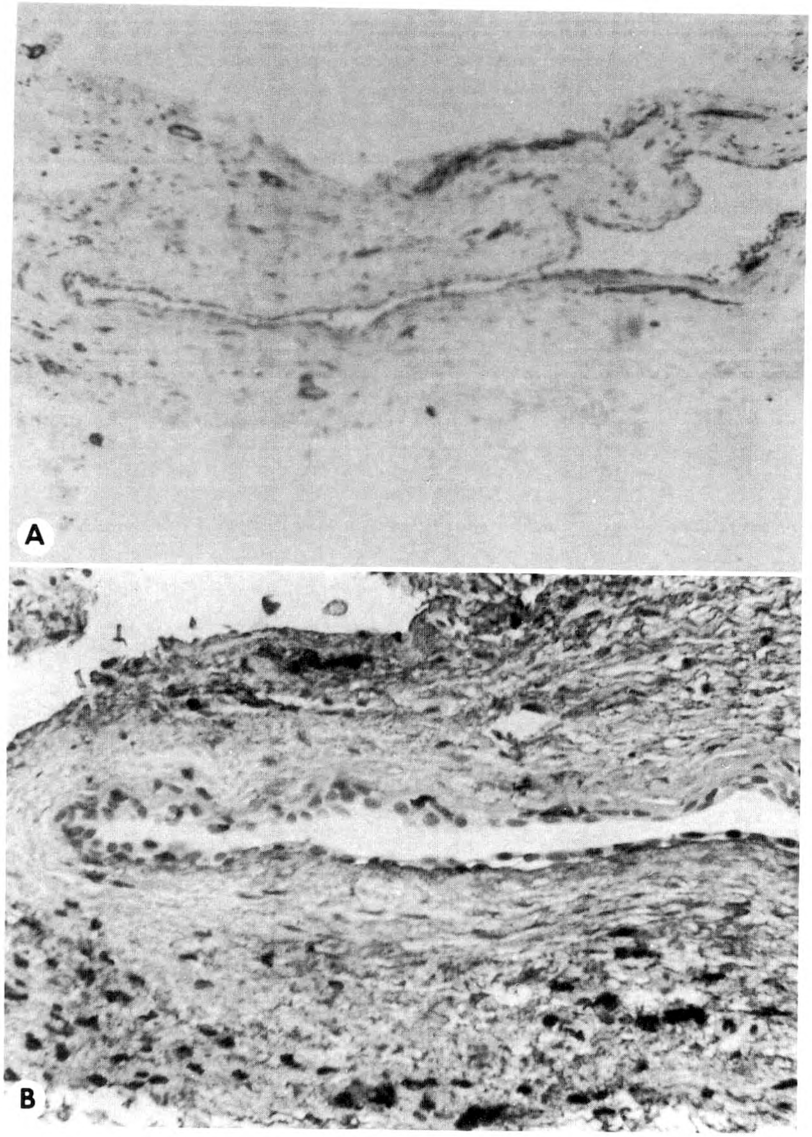

Fig 4. Mucous retention cysts. A) Of true vocal fold, removed by endoscopic microflap procedure with sparing of normal overlying vocal fold mucosa (original ×4). This is low columnar or cuboidal lining. Collagen type IV uptake is present in BMZ of cyst epithelium. B) Same specimen (original ×25). Fibronectin staining maintains laminar distribution in tissue surrounding vocal fold cyst.

duced in the BMZ of the surface epithelium when compared with collagen type IV uptake in the BMZ of the surrounding vasculature in 2 of the 4 specimens. Regarding fibronectin distribution, in 2 of the 4 specimens fibronectin maintained a general laminar distribution that was interrupted by the nodular increased uptake surrounding angiomatous clusters of vessels.

DISCUSSION

Non-neoplastic vocal fold lesions are presumed to represent a response of the vocal fold to trauma. The exact cause of these lesions and their treatment is poorly understood. Currently, vocal use modification with surgical excision for nonresponding lesions offers the most satisfactory results.[5] However, a better understanding of the extracellular matrix of the lamina propria, and the precise localization of the pathologic response, may lead to improved forms of therapy.

Collagen type IV and fibronectin are produced by fibroblasts and secreted into the extracellular matrix of human tissues. Fibronectin is a high–molecular weight glycoprotein involved in cell-to-cell adhesion, cell-to-substrate adhesion, migration and differentiation of cells, maintenance of cellular structure, and blood clotting. As such, fibronectin is involved intimately in tissue response to injury and is found in increased concentration in healing and previously injured tissue.[6,7] Therefore, fibronectin should be found in higher concentrations in vocal fold nodules and polyps, which represent a response of vocal folds to trauma.

Collagen type IV is a supporting substance of both epithelial and endothelial basement membranes. It has been localized exclusively to the lamina densa of the BMZ. This layer is normally 0.02 to 0.30 μm in width.[8] Previous experiments with hyperphonated vocal folds demonstrated widening of the BMZ in response to acute injury.[3] Therefore, in mature vocal fold lesions the BMZ may or may not be widened. Because of its localization to the lamina densa, collagen type IV serves as a good indicator of BMZ width.

Examination of our immunohistochemical staining results leads to several observations. First, specimens clinically diagnosed as nodules more commonly have a thickened BMZ and dense fibronectin arrangement when compared with lesions clinically labeled as polyps. These findings are consistent with current theories that nodules represent a response to repeated trauma that leads to BMZ thickening and a disordered fibronectin pattern secondary to attempted wound healing. Second, lesions clinically diagnosed as polyps more commonly exhibit an unaltered BMZ width, with fibronectin deposition clustered around the neovascularity. In addition, in the majority of lesions (16 of 19) clinically diagnosed as polyps, collagen type IV stained less intensely in the BMZ of the surface epithelium than it did in the BMZ of the neovasculature. Perhaps this relative lack of collagen type IV leads to weakening of the BMZ and predisposes to polyp formation. Third, these same findings were also present in 2 of the 4 specimens with polypoid corditis. The insufficient number of specimens in the polypoid corditis group, however, precludes further observations in this pathologic type of injury. Finally, lesions clinically diagnosed as cysts, either squamous inclusion or mucous retention, which may arise from vocal fold trauma or be associated with increased trauma secondary to their interruption of the glottic vibratory cycle, seem to have a BMZ thickness somewhere between that of polyps and that of nodules. Fibronectin appears to maintain a normal surrounding distribution.

CONCLUSIONS

The extracellular matrix of the human lamina propria is believed to be responsible for characteristics of vocal fold vibration. Nodules, polyps, and cysts may result from repeated trauma to this region and represent changes within the extracellular matrix. These changes disrupt the normal vibratory pattern of the human vocal fold and result in dysphonia. A better understanding of these pathologic changes may lead to more effective treatment regimens.

There are two basic patterns of injury that appear to correlate strongly with current clinical classification systems. First, lesions clinically diagnosed as nodules have an increased BMZ thickness, as well as disordered and more dense arrangement of fibronectin throughout the lamina propria. Second, lesions clinically diagnosed as polyps tend to have a more normal BMZ width. Fibronectin in these lesions is clustered around the neovasculature. Cystic lesions demonstrate yet a third pattern of response to injury. This pattern exhibits greater variability than the first two patterns. It is characterized by a normal or slightly thickened BMZ and a normal distribution of fibronectin within the lamina propria. These findings correlate with earlier published results and add to our understanding of benign pathologic responses of the larynx.

REFERENCES

1. Hirano M, Kakita Y. Cover-body theory of vocal fold vibration. In: Daniloff RG, ed. Speech science. San Diego, Calif: College-Hill Press, 1985:1-46.

2. Frenzel H. Fine structural and immunohistochemical studies on polyps of human vocal folds. In: Kirchner JA, ed. Vocal fold histopathology. San Diego, Calif: College-Hill Press, 1986: 39-50.

3. Gray SD, Titze I, Lusk RP. Electron microscopy of hyperphonated canine vocal cords. J Voice 1987;1:109-15.

4. Gray SD, Hammond E, Hanson DF. Benign pathologic responses of the larynx. Ann Otol Rhinol Laryngol 1995;104:13-8.

5. Courey MS, Ossoff RH. Surgical management of benign voice disorders. In: Gould WJ, Rubin JS, Korovin G, Sataloff R, eds. Diagnosis and treatment of voice disorders. Tokyo, Japan: Igaku-Shoin, 1995:366-82.

6. Nagata H, Ueki H, Moriguchi T. Fibronectin. Arch Dermatol 1985;121:995-9.

7. Grinnell F. Fibronectin and wound healing. J Cell Biochem 1984;26:107-16.

8. Laurie GW, Leblond CP, Martin GR. Localization of type IV collagen, laminin, heparin sulfate proteoglycan, and fibronectin to the basal lamina of basement membranes. J Cell Biol 1982; 95:340-4.

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ACUTE AIRWAY OBSTRUCTION IN THE HOSPITALIZED INFANT: FOUR HARD LESSONS IN THE DISTAL TRACHEA

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Acute obstructing lesions of the distal (lower) trachea present a unique challenge to the pediatric otolaryngologist. The small caliber of the airway and tenuous medical status of such patients test the ingenuity of the surgeon and the limits of instrumentation. This report presents the operative findings and management of four hospitalized infants with worsening respiratory distress. Difficulties encountered in maintaining airway control while diagnosing and correcting these lesions are described.

KEY WORDS — balloon catheter, extracorporeal membrane oxygenation, high-frequency jet ventilation, necrotizing tracheobronchitis, rigid bronchoscopy, tracheal obstruction, tracheal stenosis.

INTRODUCTION

The distal (lower) trachea remains a treacherous area for surgical manipulation, especially in the small airway of an infant. Advances in the resuscitation and support of premature infants have permitted the survival of ever smaller and less developed children. The use of surfactant, high-frequency jet ventilation (HFJV), and extracorporeal membrane oxygenation (ECMO) allow children born 3 months before term to survive. Long periods of endotracheal intubation, airway desiccation, and suction catheter trauma can lead to acute obstructing lesions of the distal trachea. This article will describe four difficult airway problems in critically ill infants, the endoscopic management of each, and the lessons learned from the crises.

CASE REPORTS

CASE 1: TRACHEAL OBSTRUCTION WHILE ON HIGH-FREQUENCY VENTILATION

The patient was the 1,250-g, 28-week product of a pregnancy complicated by preterm labor and rupture of membranes. Respiratory distress ensued and he could not be supported by conventional ventilation with 100% oxygen. High-frequency jet ventilation was begun. After 72 hours of successful ventilation, the servo pressure of the ventilator began to fall, indicating distal obstruction. Adequate ventilation could not be maintained despite vigorous suctioning and a change of endotracheal tubes. He was returned to conventional ventilation with 100% oxygen and high peak pressures. Despite forceful ventilation with an Ambu bag, oxygenation could not be maintained. The pediatric otolaryngology service was consulted emergently. Bedside rigid bronchoscopy with a 2.5 Jackson size, rigid rod bronchoscope revealed near-complete plugging of the distal trachea by hard mucous crusts. Direct suctioning, lavage, and the use of side-channel forceps failed to clear the tracheal obstruction. Under apneic anesthesia, the larynx was exposed with a Parsons laryngoscope and optical peanut forceps were passed into the trachea unsheathed. The obstructing plug was removed and the patient was reintubated and successfully ventilated mechanically on 100% oxygen.

Discussion. High-frequency jet ventilation has evolved in the last decade, to become an important alternative technique for the mechanical ventilation of children with respiratory distress syndrome. It employs high flow rates, making adequate humidification of inspired gases more difficult than with conventional techniques. Desiccation may lead to the formation of obstructing crusts that are firmly fixed to the tracheal wall.[1,2] Prolonged use of HFJV has been associated with acute inflammation of the tracheal wall near the tip of the endotracheal tube. This inflammation may result in loss of intraepithelial mucus and of surface cilia. In extreme cases, necrosis of epithelium can lead to extensive sloughing, airway obstruction, and death (necrotizing tracheobronchitis).[3,4] Falling servo pressure during HFJV is an early indicator that distal obstruction is occurring. Prompt bronchoscopic removal of crusts or epithelial debris can be lifesaving. Unfortunately, available optical forceps are too large to fit through bronchoscopes of less than 3.0 Jackson size. The use of naked optical forceps with apneic technique circumvents this constraint.

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CASE 2: SUBGLOTTIC VERSUS TRACHEAL STENOSIS

The patient was a 3-week-old girl, the full-term product of an uncomplicated vaginal delivery. She was noted to have inspiratory stridor at birth that worsened with agitation and feeding. She was admitted to the hospital and subsequently suffered two witnessed apneic episodes with cyanosis, one event associated with seizure activity. Plain radiographs of the neck and chest were interpreted as showing a long tracheal stenosis. A bronchogram was deemed unsafe, given her tenuous airway. After consultation with the cardiothoracic surgery service, it was decided to place the child on ECMO prior to bronchoscopy. This was intended to provide safe conditions for evaluation of the extent of the tracheal narrowing, and to allow immediate surgical correction. Under mask anesthesia, cervical cannulas were placed and ECMO was begun. The airway was visualized with a 2.5 Jackson size bronchoscope. The laryngeal and tracheal mucosa bled easily because of heparinization. A moderate subglottic stenosis was identified. There was no tracheal stenosis. A tracheotomy was performed.

Discussion. Congenital tracheal stenosis continues to carry a significant mortality despite improvements in surgical management.[5] Diagnostic workup, timing, and choice of surgical procedure remain controversial.[6] While plain radiographs can suggest tracheal stenosis, they may be deceptive in a very young child with a flexible trachea and surrounding soft tissues.[7] In the stable patient, contrast bronchography, computed tomography (CT), and magnetic resonance imaging (MRI) have specific advantages in defining the extent and severity of stenosis.[8] Each of these procedures has added risk in the unstable airway. Both MRI and CT carry the risk of airway compromise from sedation. Bronchography has a risk of frank obstruction from the contrast material.[9] Anecdotal reports of loss of airway control at bronchoscopy with tight tracheal stenoses motivated the use of ECMO in this patient. Subsequent experience has shown that careful bronchoscopy with a thin, unsheathed telescope is relatively safe[10] and would have spared this patient the risks of carotid cannulation and heparinization.

CASE 3: DISTAL GRANULATION TISSUE

The patient was a full-term girl born with trisomy 21 and a complete atrioventricular canal defect. She underwent surgical correction of the cardiac lesion at 6 months of age. Her postoperative course was complicated by sepsis and chylothorax. After 3 months of mechanical ventilation she was finally extubated, but was noted to have persistent, biphasic stridor. A bronchoscopy was performed that demonstrated a distal tracheal stenosis with a 3-mm opening, just above the carina. It was dilated with a bronchoscope, and a small endotracheal tube was placed through the stenosis and maintained in place for a week. Repeat bronchoscopy showed a near-normal airway caliber. After pretreatment with steroids, she was successfully extubated and, following a week of observation, discharged home. Nine days later she returned to the hospital with increasing respiratory distress. Direct laryngoscopy and bronchoscopy revealed recurrence of her circumferential web, now with a 2-mm lumen and adjacent granulation tissue just above the carina. A 3.5 Jackson size bronchoscope was pushed against the web. The optical field was suddenly obscured by blood and it was not possible to ventilate the patient. The bronchoscope was withdrawn and mask ventilation attempted. The patient became profoundly cyanotic and the pulse was lost. Closed chest compressions were begun and intracardiac epinephrine was administered. A 2.5 Jackson size bronchoscope was introduced and pushed blindly through the bleeding lesion, reestablishing the airway. The trachea was intubated and the patient transferred to the intensive care unit. Following a second subsequent bronchoscopic dilation, she was extubated. The distal stenosis recurred. Seven subsequent bronchoscopic dilations and a balloon dilation have been performed over a 3-year period. Resection of the stenosis is planned.

Discussion. Successful tracheal dilation with bougies or a rigid bronchoscope has been reported in adults.[11] This technique has proven less useful in the small airways of infants,[12] where longitudinal shearing of mucosa may in part account for the high rate of restenosis. In addition, bleeding from distal granulation quickly obscures vision when optical rods are used and may result in sudden loss of the airway.

CASE 4: DISTAL GRANULATION TISSUE

The patient was an 11-month-old infant who had been born prematurely (26 weeks, 940 g) and had been intubated since birth. His complications included respiratory distress syndrome, necrotizing enterocolitis and resultant short-bowel syndrome, and repeated intravenous catheter sepsis. Following the replacement of a central venous catheter under anesthesia, he was noted to have an acute worsening in respiratory status with hypoxemia and hypercarbia despite 100% inspired oxygen and bronchodilators. Rigid bronchoscopy revealed a distal tracheal web with granulation just above the carina. Removal of granulation tissue was attempted with a 3.5 Jackson size laser bronchoscope and a carbon dioxide laser. Repeated fogging of the instrument made this difficult. A Bugbee urologic electrode was then introduced through the side channel of a conventional bronchoscope, and radial incision of the web and cauterization of the granulation was performed. A 4-mm Grüntzig balloon angioplasty catheter was then introduced and inflated to 4 atm of pressure. The patient was reintubated with the endotracheal tube stenting the lesion. At follow-up bronchoscopy 7 days later, the balloon dilation was repeated. The airway remained patent, but the patient subsequently died of sepsis and intraventricular hemorrhage. Permission for autopsy was denied.

Discussion. While the carbon dioxide laser bronchoscope has proven its value in adult tracheobronchial lesions, its usefulness in the infant airway is limited. Small (3.5 Jackson size) laser bronchoscopes are now available, but have limited magnification. In addition, recurrent fogging of the proximal window is common, with resultant scatter of the laser beam and poor visualization. Contact techniques with argon, potassium titanyl phosphate, and neodymium: yttrium-aluminum-garnet lasers have been recommended to treat vascular lesions in the distal trachea.[13,14] The laser energy is delivered through a small fiber, permitting simultaneous quartz rod visualization during cautery. If such a unit is not readily available, electrocautery with a Bugbee urologic electrode works well.[15] These electrodes are insulated flexible wires with conducting ball-shaped tips. The power source is a conventional electrosurgical unit. As with fiberoptic laser techniques, a 3-mm Bugbee electrode can be passed through the side channel of even the smallest bronchoscope while an optical telescope is in place. Cautery of granulation tissue or radial incision of soft stenosis can be achieved, thus preventing the bleeding and loss of airway control seen in case 3.

Balloon dilation of pediatric tracheal stenosis has been reported in the pediatric surgery and radiology literature, with favorable results.[16-20] Angioplasty (Grüntzig, etc) rather than embolectomy (Fogarty, etc) balloon catheters should be used for dilation. Polyvinylchloride or polyethylene angioplasty balloons reach a prescribed maximum diameter, then have a continuing rise in wall tension with applied pressure. By contrast, the diameter of an embolectomy balloon continues to increase with insufflation.[21] It is the wall tension that results in radial expansion and dilation of a stricture. Both soft and hard stenoses have been treated in this fashion. Most reports describe dilation in the radiology suite following diagnosis by bronchogram. This poses some risk of airway obstruction in acute stenosis. Dilation at the time of bronchoscopy provides immediate treatment of an obstructing stenosis with sufficient airway control for management of lesions even in critically ill infants. This method has been applied to proximal bronchial lesions and to distal tracheal anastomotic sites as well.[22]

CONCLUSION

The management of obstructing lesions of the distal trachea remains a challenge. Even as improvements in endoscopic equipment and techniques to control the distal airway arrive on the scene, advances in neonatology produce unanticipated problems in ever smaller infants. It is left to the otolaryngologist to respond to these unique and dangerous lesions, often with limited time to prepare. It is hoped that the lessons taught by these four patients will help others to be ready when such crises arise.

REFERENCES

1. Chalon J, Loew DAY, Malebranche J. Effects of dry anesthetic gases on tracheobronchial ciliated epithelium. Anesthesiology 1972;237:338-43.
2. Nicklaus PJ. Airway complications of jet ventilation in neonates. Ann Otol Rhinol Laryngol 1995;104:24-30.
3. Pietsch JB, Nagaraj HS, Groff DB, Yacoub UAH, Roberts JL. Necrotizing tracheobronchitis: a new indication for emergency bronchoscopy in the neonate. J Pediatr Surg 1985;20:391-3.
4. Ophoven JP, Mammel MC, Gordon MJ, Boros SJ. Tracheobronchial histopathology associated with high-frequency jet ventilation. Crit Care Med 1984;12:829-32.
5. Hoffer ME, Tom LWC, Wetmore RF, Handler SD, Potsic WP. Congenital tracheal stenosis — the otolaryngologist's perspective. Arch Otolaryngol Head Neck Surg 1994;120:449-53.
6. Healy GB, Schuster SR, Jonas RA, McGill TJI. Correction of segmental tracheal stenosis in children. Ann Otol Rhinol Laryngol 1988;97:444-7.
7. Hernandez RJ, Tucker GF. Congenital tracheal stenosis: role of CT and high KV films. Pediatr Radiol 1987;17:197-206.
8. Blumer JR, Bauman NM, Kearns DP, Smith RJH. Distal tracheal stenosis in neonates and infants. Otolaryngol Head Neck Surg 1992;107:583-90.
9. Leoff DS, Filler RM, Vinograd I, et al. Congenital tracheal stenosis: a review of 22 patients from 1965 to 1987. J Pediatr Surg 1988;23:744-8.
10. Dunham ME, Holinger LD, Backer CL, Mavroudis C. Management of severe congenital tracheal stenosis. Ann Otol Rhinol Laryngol 1994;103:351-6.
11. Shapshay SM, Beamis JF Jr, Hybels RL, Bohigian RK. Endoscopic treatment of subglottic and tracheal stenosis by radial laser incision and dilation. Ann Otol Rhinol Laryngol 1987;96:661-4.
12. Simpson GT, Strong MS, Healy GB, Shapshay SM, Vaughan CW. Predictive factors of success or failure in the endoscopic management of laryngeal and tracheal stenosis. Ann Otol Rhinol Laryngol 1982;91:384-8.
13. Dumon J-F, Reboud E, Garbe L, Aucomte F, Meric B. Treatment of tracheobronchial lesions by laser photoresection. Chest 1982;81:278-84.

14. Azizkhan RG, Lacey SR, Wood RE. Acquired symptomatic bronchial stenosis in infants: successful management using an argon laser. J Pediatr Surg 1990;25:19-24.

15. Andrews TM, Myer CM, Cotton RT. Alternative management for laryngeal and tracheobronchial lesions: the Bugbee electrode. Otolaryngol Head Neck Surg 1990;103:841.

16. Cohen MD, Weber TR, Rao CC. Balloon dilatation of tracheal and bronchial stenosis. AJR 1984;142:477-8.

17. Groff DB, Allen JK. Gruentzig balloon catheter dilation for acquired bronchial stenosis in an infant. Ann Thorac Surg 1985;39:379-81.

18. Fowler CL, Aaland MO, Harris FL. Dilatation of bronchial stenosis with Gruentzig balloon. J Thorac Cardiovasc Surg 1987;93:308-15.

19. Lenoir P, Bougatef A, Ramet J, Dab I, Sacre L. Nonsurgical intervention in the resolution of acquired neonatal bronchial stenosis. Am J Perinatol 1990;7:290-2.

20. Hebra A, Powell DD, Smith CD, Othersen HB. Balloon tracheoplasty in children: results of a 15 year experience. J Pediatr Surg 1991;26:957-61.

21. Hall DP, Gruentzig AR. Technique of percutaneous transluminal angioplasty of coronary, renal, mesenteric and peripheral arteries. In: Hurst JW, ed. The heart. 6th ed. New York, NY: McGraw-Hill Book Co, 1986:1901-15.

22. Skedros DG, Chan KH, Siewers RD, Atlas AB. Rigid bronchoscopy balloon catheter dilation for bronchial stenosis in infants. Ann Otol Rhinol Laryngol 1993;102:266-70.

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ACOUSTICAL SOCIETY OF AMERICA AND ACOUSTICAL SOCIETY OF JAPAN

The Third Joint Meeting of the Acoustical Society of America and the Acoustical Society of Japan will be held December 2-6, 1996, in Honolulu, Hawaii. For further information, contact Acoustical Society of America, 500 Sunnyside Blvd, Woodbury, NY 11797; telephone (516) 576-2360; fax (516) 576-2377.

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EPITHELIAL HYPERPLASTIC LESIONS OF THE LARYNX

The International Conference on Epithelial Hyperplastic Lesions of the Larynx will be held October 28-30, 1996, in Ljubljana, Slovenia. For more information, contact Bostjan Luzar, MD, or Aleksandar Vodovnik, MD, Institute of Pathology, Korytkova 2, 61105 Ljubljana, Slovenia; telephone 386 61 1403042 (Int. 268); fax 386 61 301 816; E-mail Vodovnik@IBMI.MF.UNI-LJ.SI.

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OMOHYOID MUSCLE TRANSPOSITION FOR THE TREATMENT OF BOWED VOCAL FOLD

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Imperfect glottal closure is usually the most important factor causing dysphonia in patients with bowing of the vocal folds. We have performed laryngeal framework surgery, which allows the medialization of the vocal folds from the outside without creating any scar tissue on them. Over the past 6 years, however, we encountered three cases with marked bowing of the vocal folds that could not be cured by laryngeal framework surgery alone. We used an open laryngeal procedure in these cases, even though such procedures had been considered contraindicated in the treatment of hoarseness. After performing a laryngofissure, we made a small pocket beneath the vocal fold mucosa at the anterior commissure. The superiorly based omohyoid muscle flap was then transposed into the mucosal pocket and sutured to the vocal process. This procedure should be considered an option in treating highly bowed vocal folds.

KEY WORDS — atrophy, bowing, larynx, omohyoid muscle, vocal fold.

INTRODUCTION

Bowing of the vocal folds is observed in patients with vocal fold paralysis, in patients with vocal fold atrophy with or without a sulcus, and in patients with a defect in the vocal fold after laryngeal trauma. Imperfect glottal closure is usually the most important factor causing dysphonia in such patients. We have performed laryngeal framework surgery, which allows the medialization of the vocal folds from the outside without creating any scar tissue on them. The results were excellent in patients with vocal fold paralysis, and good in those with vocal fold atrophy. However, there have been some cases in which laryngeal framework surgery was ineffective, and no other means were available to treat the hoarseness. In phonosurgery, the first priority is to avoid scar formation within the vocal fold. Therefore, open laryngeal procedures, including muscle transposition, had been considered contraindicated in the treatment of hoarseness.

Various surgical procedures exploiting muscle transposition have been reported,[1-4] and have been used successfully to compensate for the loss of bulk following a partial laryngectomy. Thus, muscle transposition seems to have established a definite role in laryngeal reconstruction. However, it was limited in application to reconstruction after a tumor resection. We intended to utilize omohyoid transposition for the treatment of extremely bowed vocal folds, and applied it to a case of laryngeal trauma in 1989. Since then, we have used this procedure in three other cases. In the most recent case, which is the focus of this paper, we used this procedure for idiopathic vocal fold atrophy. On the basis of the follow-up results, its indication and applicability in comparison with thyroplasty type I are discussed.

SURGICAL PROCEDURE

A tracheotomy was performed under local anesthesia, and an endotracheal tube was placed for general anesthesia. A skin incision was made over the thyroid cartilage. (It can be either horizontal or vertical, but should be kept separate from the tracheostomy incision.) The skin flaps, including the platysma muscles, were elevated, and the lower edge of the hyoid bone above and the cricoid cartilage below were exposed. The fascia of the strap muscles was incised in the midline to expose the thyroid cartilage. After retraction of the strap muscles laterally, the thyroid cartilage was carefully incised in the midline without entering the lumen of the larynx at this time. (In some cases, a fine bur may be required for cutting the calcified cartilage.) After a small initial incision was made on the inner perichondrium above the glottis to survey the inside of the larynx, the anterior commissure was transected in its exact center. A small portion of the thyroid cartilage was removed near the anterior commissure of the affected side, and a tiny incision was made beneath the vocal fold. Submucosal elevation from the anterior commissure to the level of the vocal process was employed to

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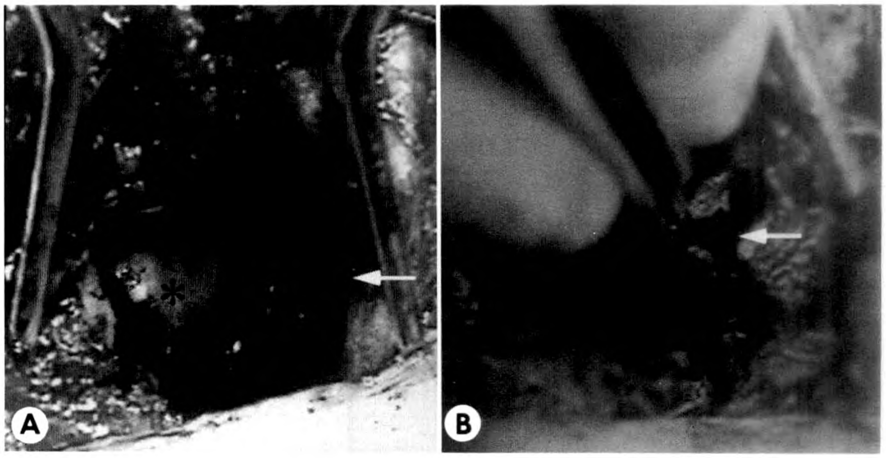

Fig 1. (Case 3) A) Intraoperative laryngeal findings after thyrotomy showing no left vocal prominence (arrow). Asterisk — right vocal fold. B) Pocket (arrow) for omohyoid muscle transposition.

create a pocket in the affected side of the larynx (Fig 1).

After completion of the pocket in the vocal fold, the sternocleidomastoid muscle was retracted laterally to expose the omohyoid muscle, which was dissected inferiorly and transected. Its cut end was rotated and transposed into the submucosal pocket of the larynx, which had already been prepared, and fixed to the vocal process with 5-0 Vicryl sutures. Since the mucosa of the vocal fold was completely preserved, a laryngeal keel was not necessary. However, special attention must be paid to the precise approximation of the mucosa at the anterior commissure of the larynx.

The laryngeal mucosa was approximated with simple interrupted 5-0 Vicryl sutures, and then the larynx was closed by suturing the external perichondrium with interrupted 4-0 nylon sutures (Fig 2).

SUBJECTS

We have performed this omohyoid muscle transposition procedure in three cases (see Table) for the treatment of bowed vocal folds. Each patient complained of breathy or weak phonation. The bowed vocal fold was posttraumatic in case 1, post–laser operation in case 2, and idiopathic in case 3. Except for case 2, the prominence of the affected side of the vocal fold totally disappeared, but the lesion was limited to one vocal fold. In case 2, the prominence of the left vocal fold was retained, but was highly bowed because of the previous laser surgery. Moreover, a scar created by the laser surgery was observed on the right vocal fold, as well as the anterior commissure. Since atrophic lesions were observed on both sides of the vocal folds, thyroplasty type I as an additional procedure was performed in case 2.

From the viewpoint of etiology, case 3 was different from the others. In this case, there were no scars on the vocal folds that could have been due to surgical or traumatic intervention before the present treatment. The detailed clinical history of this patient is as follows. This 55-year-old man was referred to our hospital complaining of hoarseness. He had suffered a breathy voice since he was 14 years old. Fiberscopic findings showed severe atrophy of the left vocal fold, which caused remarkably insufficient glottal closure. The maximum phonation time (MPT) was 5 seconds and the mean flow rate (MFR) was 568 mL/s. For the purposes of improving hoarseness, Isshiki's thyroplasty type I was performed. However, the postop-

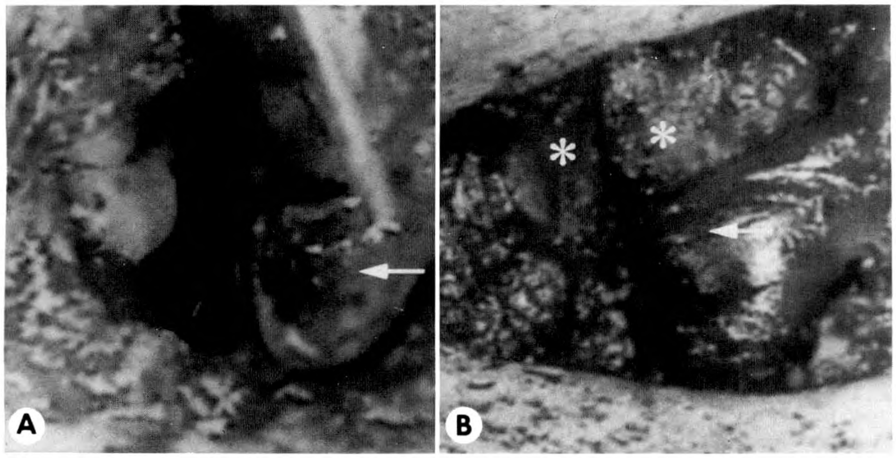

Fig 2. (Case 3) A) Omohyoid muscle (arrow) inserted into pocket. B) Laryngeal framework findings after closure of thyroid cartilage. Asterisks — both fragments of thyroid cartilage, arrow — omohyoid muscle.

PATIENT DATA

Case	Age (y)	Sex	Side of Atrophy	Cause
1	26	Male	Left vocal fold	Posttraumatic
2	54	Male	Both vocal folds	Post–laser operation
3	55	Male	Left vocal fold	Idiopathic

erative results showed no significant improvement (Fig 3). In order to create the left vocal prominence, we then performed this omohyoid flap technique 2 years after the initial thyroplasty type I.

RESULTS

The surgical effect was noted in ease of phonation rather than in voice quality in each case. It was evaluated by the measurement of the MFR and the MPT. Figure 4 shows the results of the postoperative phonatory examinations. In case 1, the phonatory examination after the omohyoid muscle transposition showed remarkable improvement. The MPT was extended from 15 seconds to 34 seconds, and the MFR decreased from 430 mL/s to 123, by 2 months after the operation. This was largely due to the increased prominence of the left vocal fold. Case 2 was first treated with omohyoid muscle transposition alone, which resulted in little improvement in the voice. The MPT was extended slightly, from 3 seconds to 6, and the MFR had changed from 368 to 353 mL/s by 1 year after the operation. With the addition of a thyroplasty type I, the MPT was extended to 10 seconds and the MFR decreased slightly, to 333 mL/s, by 1 year after the thyroplasty. In case 3, the MPT showed a minor increase, from 4 seconds to 7, and the MFR changed from 568 mL/s to 471 by 10 months after the thyroplasty. The omohyoid muscle transposition improved the MPT to 28 seconds and the MFR to 158 mL/s, by 6 months after this operation. Figure 5 shows the laryngeal findings in case 3 as seen 5 months after the surgery. A satisfactory prominence was observed at the left vocal fold, with sufficient glottal closure. According to subjective acoustic impressions, the breathy element of the patient's voice had also disappeared. No evidence of atrophy of the omohyoid flap has been seen.

DISCUSSION

Two major procedures, intracordal injection[5-12] and framework surgery,[13,14] are widely used to treat dysphonia in most cases with bowing of the vocal folds. The advantages of intracordal injection are its surgical simplicity, and with the introduction of collagen[5-8] or fat,[11,12] the safe and satisfactory results obtained in most cases. In contrast, framework surgery has the disadvantage of creating a cervical scar due to the skin incision. However, it has two crucial advantages: 1) it allows for medialization of the vocal folds without creating any scar tissue on them, and 2) the voice quality can be ascertained during the procedure and thus there are very few failures.

In our institution, we have chosen laryngeal framework surgery more frequently since the early 1970s because of its reliability. The results were excellent in patients with vocal fold paralysis, and good in those with vocal fold atrophy. Over the past 6 years, however, we encountered three cases that could not be cured by laryngeal framework surgery alone. In each case, marked bowing of the vocal folds due to laser surgery, laryngeal trauma, or idiopathic atrophy was observed. This caused the prominence of the affected vocal fold to be too low, and thus it could not be medialized by medial displacement of the window cartilage in thyroplasty type I. We used an open laryngeal procedure in these cases, even though such procedures had been considered contraindicated in

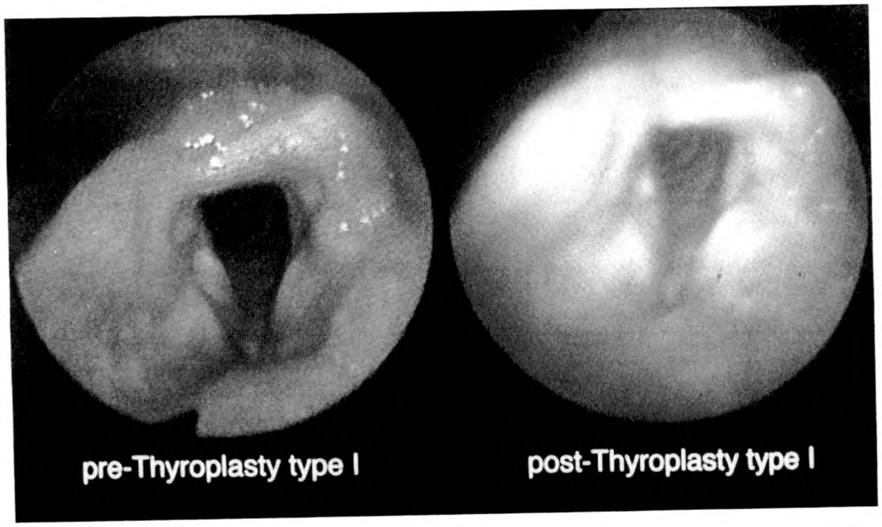

Fig 3. (Case 3) Endoscopic laryngeal findings before and after thyroplasty type I. Preoperative findings show severe atrophy of left vocal fold. Postoperative findings show little increase of left vocal prominence 10 months after thyroplasty.

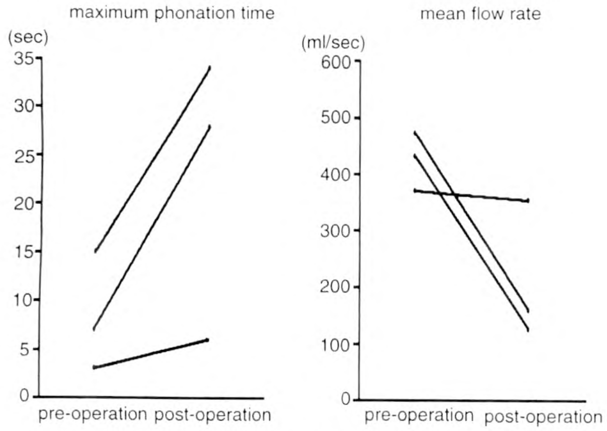

Fig 4. Maximum phonation time and mean flow rate in three patients before and after omohyoid muscle transposition.

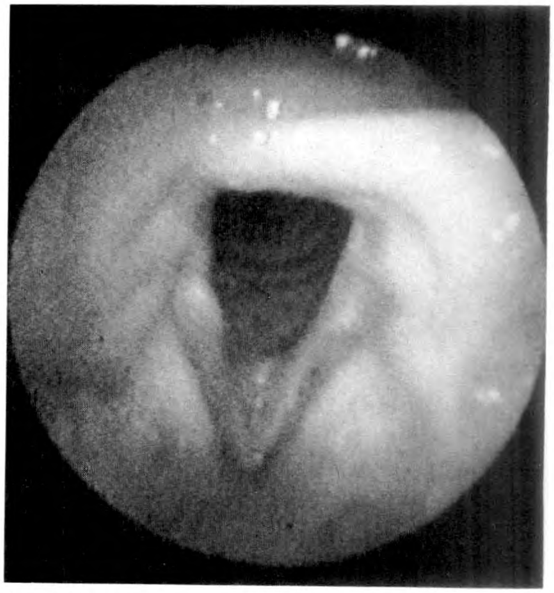

Fig 5. (Case 3) Endoscopic laryngeal findings 5 months after omohyoid muscle transposition. Adequate prominence of left vocal fold is observed.

the treatment of hoarseness.

Of the various surgical procedures used to compensate for the loss of bulk following a partial laryngectomy, the use of muscle flaps has been established as a definitive procedure in laryngeal reconstruction. Calcaterra[3] applied bilateral omohyoid muscle flaps for reconstruction following anterior commissure cancer, and concluded that this was an ideal flap for repairing small defects. Since the size of this flap was considered suitable for the reconstruction of the vocal folds, we utilized it in the treatment of the bowed vocal folds. It provides a sufficient bulge, resulting in good correction of the glottal closure as well as amelioration of the mass imbalance between the two vocal folds. Since the aim of the operation was to restore vocal quality, we were careful not to damage the mucosa of the larynx, especially on the vocal folds. To create the prominence of the vocal fold while preserving the mucosa, we made a pocket beneath the vocal fold mucosa, and inserted the omohyoid muscle flap into it. We also paid special attention to the approximation of the mucosa at the anterior commissure. As a result, the vocal fold, including the anterior commissure, was reconstructed to its normal shape without use of a stent in any case.

From the viewpoint of phonatory function, better surgical results were obtained for ease of phonation than for voice quality. Measurements of the MFR and the MPT were performed to assess it. The results obtained on phonation tests were excellent in case 1, but unsatisfactory in case 2. The cause of the hoarseness was the same; both patients had an injury that induced scar formation on the vocal folds. However, the extent of the lesions was different between the two cases. In case 2, scars and atrophic lesions were observed on both sides of the vocal folds, which had been created by the previous laser surgery. This was the primary reason why case 2 required additional surgery (thyroplasty I) to obtain complete glottal closure. However, the results after thyroplasty I in case 2 did not show sufficiently good phonation in comparison to case 1. Although complete glottal closure can be achieved by thyroplasty I, the vibratile nature of the vocal mucosa cannot be restored once it becomes scarred. The loss of normal vibration of the vocal folds resulted in poor phonatory function in case 2, whereas good phonatory function was retained in case 1 because of the mucosal vibration on the intact side of the vocal folds.

We applied the omohyoid muscle transposition procedure to the patient in case 3, who had idiopathic atrophic vocal folds on which no scar lesions were found before the treatment. We usually select framework surgery for such cases because it allows for complete glottal closure from the outside without creating any scar tissue on the vocal folds. Therefore, thyroplasty I was initially performed in this case. The results were disappointing, and it was concluded that the vocal fold was not prominent enough to be medialized by the framework surgery. The relationship between the framework and the soft tissues of the larynx is analogous to that of a container and its contents; an adequate volume of soft tissues in proportion to the framework is necessary to obtain good phonation. In the case of vocal atrophy, the volume of the soft tissues would be a little less than the framework requires. Thyroplasty I is designed to diminish the size of the container, aiming to restore the proper balance between the size of the container and its contents. However, thyroplasty I has definite limitations, and a different strategy should be considered in the correction of excessive imbalances. Therefore, we believed that increasing the volume of the contents could resolve this problem. At this point, we had assumed that there were no means available except for open laryngeal surgery to treat this case. We performed an omohyoid muscle transposition in the same manner as in cases 1 and 2, preserving the mucosa of the vocal folds.

In case 3, we expected to restore normal mucosal vibration on the reconstructed vocal fold, because no scars were found before the transposition procedure. Unexpectedly, stroboscopic findings after the surgery revealed no vibrations on the reconstructed fold. The site of the pocket created for the muscle transposition may have been too shallow on the surface of the mucosa, thus causing scar formation on the vocal fold. To avoid damaging the mucosa, the pocket can be created in the muscle layer of the vocal fold.

The majority of cases with vocal fold bowing may be treated well by the conventional procedures; this has prevented us from obtaining more clinical experience with our procedure with longer follow-up. The number of cases presented in this paper is not large enough to draw final conclusions about this procedure, and moreover, some improvements in the future may extend its applicability in the field of phonosurgery. It is, however, believed that this procedure may prove remarkably useful in treating highly bowed vocal folds and could provide an additional option that had been considered contraindicated in phonosurgery. In combination with thyroplasty I, this could play an important role in the treatment of hoarseness due to imperfect glottal closure.

REFERENCES

1. Calcaterra TC. Sternohyoid myofascial flap reconstruction of the larynx for vertical partial laryngectomy. Laryngoscope 1983;93:422-4.
2. Pressman JJ. Cancer of the larynx. Laryngoplasty to avoid laryngectomy. Arch Otolaryngol 1954;59:395-412.
3. Calcaterra TC. Bilateral omohyoid muscle flap reconstruction for anterior commissure cancer. Laryngoscope 1987;97: 810-3.
4. Lore JM Jr. An atlas of head and neck surgery. Philadelphia, Pa: WB Saunders, 1988:908-9.
5. Remacle M, Clerin M, Dubois P, Ryckaert M, Bertrand B, Hamoir M. Exploration of glottic function before and after injection of collagen for rehabilitation of the vocal cord. Acta Otorhinolaryngol Belg 1986;40:405-20.
6. Remacle MJM, Marbaix E, Bertrand BMG. The value of injectable collagen in vocal and glottic rehabilitation. Arch Otorhinolaryngol 1986;243:233-7.
7. Ford CN, Bless DM, Loftus JM. Role of injectable collagen in the treatment of glottic insufficiency: a study of 119 patients. Ann Otol Rhinol Laryngol 1992;101:237-47.
8. Ford CN, Bless DM. Clinical experience with injectable collagen for vocal fold augmentation. Laryngoscope 1986;96:863-9.
9. Lewy RB. Teflon injection of the vocal cord: complications, errors, and precautions. Ann Otol Rhinol Laryngol 1983;92: 473-4.
10. Ward PH, Hanson DG, Abemayor E. Transcutaneous Teflon injection of the paralyzed vocal cord: a new technique. Laryngoscope 1985;95:644-9.
11. Hill DP, Meyers AD, Harris J. Autologous fat injection for vocal cord medialization in the canine larynx. Laryngoscope 1991;101:344-8.
12. Mikaelian DO, Lowry LD, Sataloff RT. Lipoinjection for unilateral vocal cord paralysis. Laryngoscope 1991;101:465-8.
13. Isshiki N, Okamura H, Ishikawa T. Thyroplasty type I (lateral compression) for dysphonia due to vocal cord paralysis or atrophy. Acta Otolaryngol (Stockh) 1975;80:465-73.
14. Blaugrund SM, Taira T, El-Assuooty A, Lin P-T, Isshiki N, Gould WJ. Effects of lateral manual compression upon glottic incompetence: objective evaluations. Ann Otol Rhinol Laryngol 1990;99:249-55.

Ann Otol Rhinol Laryngol 105:1996

MANAGEMENT OF SUBGLOTTIC FOREIGN BODIES

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Foreign body aspiration is not an infrequent encounter in the practice of otolaryngology and requires immediate attention. The vast majority of foreign body aspirations occur in children less than 3 years of age, and the actual event of aspiration is frequently not witnessed. Although inhaled foreign bodies most often lodge in the bronchi, laryngotracheal foreign bodies also occur and are potentially more dangerous. Specifically, subglottic foreign bodies present unique clinical challenges. The diagnosis of subglottic foreign bodies is often difficult and they are commonly confused with other causes of upper airway obstruction. We present our experience with the diagnosis and management of seven patients with subglottic foreign bodies, who presented with an abnormal airway and whose problems were initially misdiagnosed. The radiographic and clinical features are discussed with a review of our surgical management.

KEY WORDS — aspiration, foreign body, management, subglottic foreign body.

INTRODUCTION

The presentation of a foreign body of obstructing size within the larynx often results in immediate mortality. Fortunately, most choking victims are able to spontaneously cough out the obstruction. Smaller objects, however, may be aspirated into the lower respiratory tract and produce characteristic symptoms. Rarely, aspiration of a foreign body may result in the object's lodging in the larynx or subglottis. Nonlethal aspiration of a foreign body in the larynx varies in the reported incidence of all aspirated foreign bodies from 2% to 12%.[1] Unfortunately, this unique presentation is frequently not recognized, and appropriate management is usually delayed by the misdiagnosis.[2-5]

No study to date has addressed subglottic foreign body aspiration in detail or reviewed a series of patients. We review a series of seven patients who presented with a clinically abnormal airway and were found to have a subglottic foreign body. The study attempts to identify clinical and radiographic findings that alert the physician to an aspirated subglottic foreign body.

MATERIALS AND METHODS

A retrospective review of the medical records of all children who presented with a laryngotracheal foreign body to the Medical College of Georgia between 1980 and 1992 was undertaken. Only those cases of subglottic foreign body demonstrated during endoscopy were included for discussion. The following factors were identified prior to review: age in months, patient's sex, patient's race, general health prior to aspiration, presenting symptoms and diagnosis on admission, history of witnessed aspiration, duration of symptoms prior to correct diagnosis, initial therapy and clinical response, radiographic findings, nature of obstructing object, endoscopic findings, and complications.

RESULTS

One hundred fourteen children presented to the Medical College of Georgia with a laryngotracheal foreign body. Seven children were found to have a subglottic foreign body, for an incidence of 6.1% of all laryngotracheal foreign bodies at our institution. The clinical features of the patients are reviewed in Table 1. Of the seven children, five were boys and two were girls. The ages ranged from 8 months to 7 years of age, with a median age of 10 months. All children were in good general health prior to the aspiration event. Presenting symptoms are summarized in Table 2. The symptoms on initial presentation varied and included stridor, wheezing, cough, hoarseness, and brief periods of cyanosis.

The interval between onset of symptoms and endoscopic diagnosis ranged from 1 day to 4 months. Initial diagnoses included croup, asthma, bronchitis, and vocal cord polyp. Radiographic evaluation of the airway was performed in five patients. The other two patients were those with the longest interval between

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TABLE 1. SUBGLOTTIC FOREIGN BODIES

Patient	Age (mo)	Sex	Race	Clinical Presentation	Initial Diagnosis	Prediagnosis Interval	Foreign Body
1	9	M	B	Wheezing, cough	RAD	6 d	Sandspur
2	9	M	B	Wheezing, stridor	Croup	10 d	Portion of ballpoint pen
3	11	F	W	Stridor, cyanosis	Croup	2 mo	Bacon wrapper
4	8	M	W	Stridor	Croup, bronchitis	2 d	Eggshell
5	10	M	B	Cough, cyanosis	Choking episode	1 d	Piece of plastic
6	36	F	B	Hoarseness	Vocal cord polyp	3 mo	Thin wire, 3.5 cm long
7	84	M	W	Cough, bloody sputum, wheezing, hoarseness	Possible foreign body aspiration	4 d	Sandspur

B — black, W — white, RAD — reactive airway disease.

onset of symptoms and eventual endoscopic discovery of the foreign body. Retrospective analysis of serial chest radiographs in these two patients did identify subtle subglottic narrowing in one.

Foreign body aspiration was immediately suspected in only one patient secondary to a witnessed event, but the child was not referred for medical attention until bloody sputum and hoarseness developed. All of the patients had wheezing and stridor minimally responsive to antibiotics and bronchodilators. Four patients had moderate improvement of symptoms with antibiotics, although persistent stridor and wheezing were present. Four patients were incorrectly judged to have croup or asthma. Only after consultation with an otolaryngologist and appropriate radiographic evaluation was the correct diagnosis determined.

The treatment for all patients was endoscopic removal under general anesthesia. All patients had endoscopy with the patient apneic without intubation. All patients had a foreign body identified in the subglottic area at the time of endoscopy. Two patients, both of whom were symptomatic for greater than 2 months, had significant granulation tissue formation around the foreign body. After endoscopic removal, all patients were electively intubated for control of the airway and prevention of laryngospasm. In addition, all patients received perioperative and immediate postoperative intravenous steroids. No complications occurred at the time of endoscopy or during postoperative hospitalization.

TABLE 2. PRESENTING SYMPTOMS

Symptom	Patients No.	Patients %
Stridor	5/7	71
Wheezing	4/7	57
Cough	3/7	43
Cyanosis	2/7	29
Hoarseness	2/7	29

Patients presented with more than one symptom.

DISCUSSION

Foreign bodies within the laryngotracheal region are rare and pose life-threatening emergencies when encountered. The vast majority of foreign bodies in this region lodge in the supraglottis or larynx. Aspiration of objects large enough to obstruct the supraglottis or glottis is usually life-threatening and not a diagnostic dilemma. However, the diagnosis becomes a challenge when the object passes through the glottis and lodges in the subglottis. Airway obstruction of various degrees is usually evident on presentation, but when the aspiration event has not been witnessed, the cause is often difficult to discern. The reported incidence of witnessed events ranges from 73% to 85% of all patients who aspirate; however, many recollections were anecdotal and arose after the diagnosis had been established.[6]

The initial response to a laryngotracheal foreign body includes respiratory distress, paroxysms of coughing, choking, wheezing, and stridor. Brief periods of cyanosis may occur due to periods of laryngospasm.[7] The initial response is then followed by an asymptomatic interval as the foreign body lodges and the reflexes become fatigued. This apparent symptomatic improvement results in the delay in the correct diagnosis of unrecognized foreign bodies. Protection of the airway from a foreign body is dependent on the flow of a fast-moving column of air exerting pressure over the object's surface during the act of coughing.[5] The presence of small foreign bodies lodged in the subglottis may be partially explained by this protective reflex and the anatomic shape of the subglottic region. Following aspiration, the object may be inhaled into the trachea or lower respiratory tract. When the cough reflex initiates a fast-moving column of air, the object may be lifted and propelled against the undersurface of the true vocal cords in the region created by the conus elasticus.

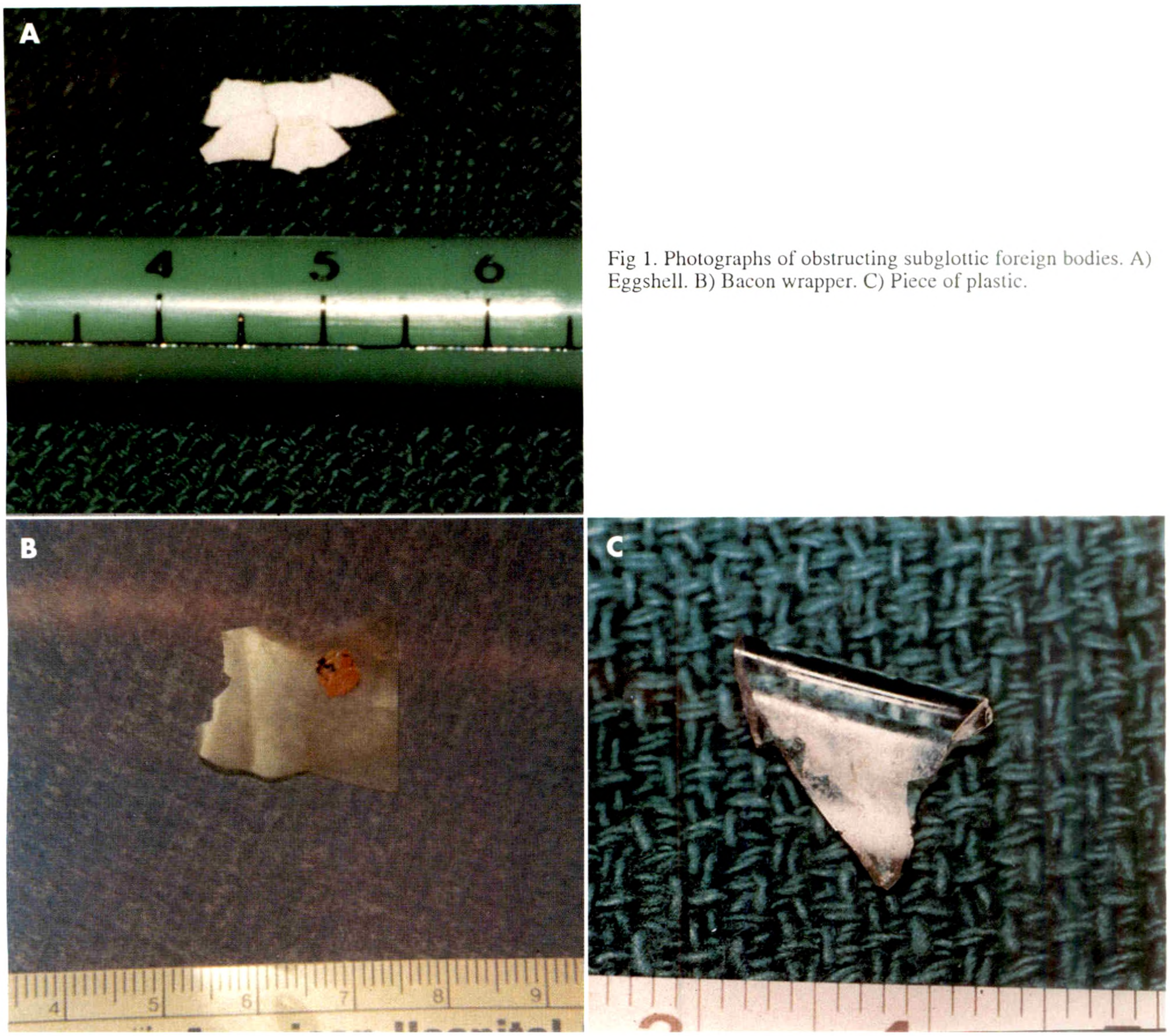

Fig 1. Photographs of obstructing subglottic foreign bodies. A) Eggshell. B) Bacon wrapper. C) Piece of plastic.

All of the objects in our study were thin, with sharp edges (Fig 1). Sharp objects may easily become embedded, and thin, flat objects may be trapped in the subglottic region unless the object exits in the sagittal plane. The unique features characterizing subglottic foreign bodies are a size small enough to pass through the glottis and sharp enough edges to embed in the subglottis.

The present study indicates that a subglottic foreign body is a diagnostic challenge, and the majority of patients had a delay in diagnosis. The correct diagnosis in all seven patients was made only after persistent symptoms or significant local response. Each patient had an episode of stridor that was not recognized as related to the subglottic foreign body. Unfortunately, the eventual diagnosis may require significant local response to the foreign object before it can be established. Two of the seven patients had significant granulation tissue formation in the subglottis and were the patients with the greatest delays in diagnosis. In addition, only one patient had a history of aspiration (but did not seek medical attention until 4 days after the event). Protracted episodes of stridor, croup, or hoarseness should raise the possibility of a subglottic foreign body.

In all patients with airway radiographs, definitive or suggestive evidence of a subglottic narrowing was found. The presentation of a thin, radiopaque lucency in the sagittal plane within the subglottic region is highly suggestive of a foreign body. Of interest, one patient had only mild subglottic narrowing in the lateral view, but a foreign body was identified in the anterior-posterior plane (Fig 2).

Under controlled circumstances and in coordination with an anesthesiologist, each patient was submitted to general anesthesia and rendered apneic prior to endoscopy. The endoscopy was performed

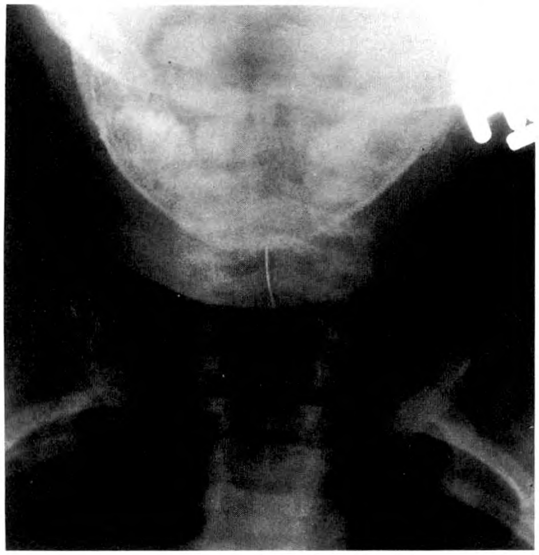

Fig 2. Endoscopy revealed eggshell lying in anterior-posterior direction. Linear nature of eggshell in this direction allowed for sufficient calcification to be demonstrated on radiograph that correct diagnosis could be established.

without the patient intubated; this technique afforded complete visualization of the immediate subglottis and avoided the possibility of dislodging the foreign body with the endotracheal tube. After removal of the foreign body, each patient was intubated and then extubated in the operating room after all general anesthesia was discontinued. This was done to prevent laryngospasm and control the airway during this critical period. None of the patients required reintubation, and each child had a normal airway and voice 6 months after the aspiration event. According to our findings, removal can be relatively safely performed in a controlled environment without significant intraoperative or postoperative sequelae.

Several large series have presented nonlethal obstruction of the larynx, although the subglottic region was not specifically addressed in any of the studies.[6,8,9] This present study identifies a number of important factors unique to subglottic foreign bodies. The first is that all patients had a delay in diagnosis of a subglottic foreign body. Second, proper radiographic evaluation aided significantly in the evaluation and aided in the diagnosis. In our series, all patients with proper diagnostic imaging had radiographic evidence of a subglottic foreign body. Additionally, patients with subglottic foreign bodies of a nonlethal size tolerated the foreign body without acute life-threatening airway compromise. This feature also complicates diagnosis, as the presentation of wheezing and stridor does not always point to the subglottis as the site of obstruction. Nonetheless, until proven otherwise, a subglottic foreign body should always be considered when airway compromise is evident.

Although our series identifies many key factors, clinical suspicion is usually the most important in determining the presence of a foreign body. A thorough history from the patient and parents, physical examination, and radiographic evaluation are crucial to diagnosis. The reappearance of symptoms or continued airway compromise should alert the physician to the possibility of foreign body aspiration. The lack of a history of foreign body aspiration should not exclude it from the differential diagnosis.

In conclusion, a subglottic foreign body is a unique presentation of aspiration and presents a challenge to the practitioner. Proper evaluation and sound clinical judgment will often diagnose a subglottic foreign body.

REFERENCES

1. Lima JA. Laryngeal foreign bodies in children: a persistent, life-threatening problem. Laryngoscope 1989;99:415-20.

2. Gyi B, Austin JHM, Eisenberg LD. Radiolucent intratracheal foreign body mistaken for croup in a 9-year-old boy. Am J Dis Child 1984;138:749-50.

3. Caglayan S, Erkin S, Loteli I, et al. Bronchial foreign body v. asthma. Chest 1989;96:509-11.

4. Moskowitz D, Gardiner CJ, Sasaki CT. Foreign body aspiration. Arch Otolaryngol 1982;108:806-7.

5. Esclamado RM, Richardson MA. Laryngotracheal foreign bodies in children. Am J Dis Child 1987;141:259-62.

6. Rothmann BF, Boeckman CR. Foreign bodies in the larynx and tracheobronchial tree in children. A review of 225 cases. Ann Otol Rhinol Laryngol 1980;89:434-6.

7. Banerjee A, Rao KS, Khanna SK, et al. Laryngo-tracheobronchial foreign bodies in children. J Laryngol Otol 1988;102: 1029-32.

8. Daniilidis J, Symeonidis B, Triaridis K, et al. Foreign body in the airways. A review of 90 cases. Arch Otolaryngol 1977;103:570-3.

9. Cohen SR, Herbert WI, Lewis GB Jr, Geller KA. Foreign bodies in the airway. Five-year retrospective study with special reference to management. Ann Otol Rhinol Laryngol 1980;89: 437-42.

Ann Otol Rhinol Laryngol 105:1996

VIDEOENDOSCOPIC ANALYSIS OF LARYNGEAL FUNCTION DURING LAUGHTER

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Although commonly encountered in all human cultures, laughter remains poorly understood. In order to examine laryngeal function during laughter, telescopic and fiberscopic videolaryngoscopy was performed on five subjects, who laughed in the different vowels, at various frequencies, and in several voice qualities. During laughter, the vocal folds were found consistently to undergo rhythmic abduction and adduction. At the end of these specific phonation tasks, all subjects were able to gain voluntary control of paramedian vocal fold positioning. This study better defined laryngeal function during laughter. These results have important clinical implications. Voluntary vocal fold positioning has important applications in speech therapy for dysphonias, such as vocal fold nodules, in which the primary cause is vocal fold hyperadduction. Patients suffering from these hyperadductive dysphonias may be able to utilize laughter to correct them.

KEY WORDS — laughter, stroboscopic videolaryngoscopy, videolaryngoscopy, voice therapy.

INTRODUCTION

Dorland's Illustrated Medical Dictionary defines laughter as "as series of spasmodic and partly involuntary expirations with inarticulate vocalization, normally indicative of merriment, often a hysteric manifestation or a reflex result of tickling."[1(p899)] Consequently, laughter may be considered a stereotypic response to physical stimuli (such as tickling) or psychologic concepts (such as humor). The complexity of laughter reflects its dual nature as a reflex and as a psychosomatic event.[2] Many different stimuli, both physical and psychologic, may produce laughter, and the impact of these stimuli on different individuals may vary. Despite the wide spectrum of these stimuli and their individual interpretations, all laughter, once initiated, is produced by a single mechanism, which encompasses both the vocal and respiratory tracts.

Despite its ubiquity in all human cultures and societies, laughter remains poorly understood. Studies of laughter may focus on the social context of laughter, as well the individual's cognitive interpretation of stories or events. In the central nervous system, the cerebral cortex, the limbic system, and brain stem nuclei, as well as their interconnections, are all involved in the initiation of laughter. Laughter, however, is an end product of a single effector system that produces changes in the vocal and respiratory tracts, as well as in the cardiovascular and the musculoskeletal systems. Scientific inquiries into laughter may assess any of these potential aspects.

Because the sound of laughter is one of its most central characteristics and because so little is known about the vocal tract mechanisms that produce this sound, this study was undertaken to examine the role of the larynx in generating laughter. By means of both fiberscopes and telescopes, the larynx was observed during laughter. These observations have important implications for the therapy of hyperadductive dysphonias.

METHODS

Five professional singers were recruited for the study. The group included two men and three women, whose ages ranged from 32 to 46 years. Each subject was given the protocol in advance so that he or she had the opportunity to practice the specific exercises.

The protocol was divided into two parts. Part 1 involved three distinct tasks. After the intranasal application of topical 4% lidocaine, each subject was asked to laugh in each vowel sound (/i/, /e/, /a/, /o/, and /u/). For the second task, each subject laughed at high, low, and normal frequencies. Last, each subject laughed in the different voice qualities (speech, falsetto, sob, twang, opera, and belting).[3-5] During each task, telescopic and fiberscopic videolaryngoscopy was performed, and each subject was able to view these images on a nearby video monitor as he or

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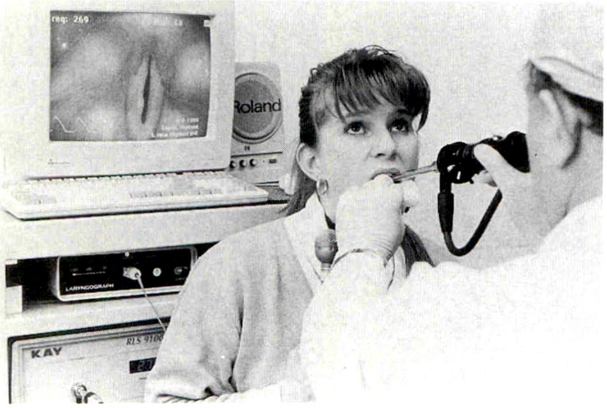

Fig 1. Technique of telescopic stroboscopic videolaryngoscopy.

she performed each phonation task.

At the conclusion of these three tasks, each subject proceeded directly to the second portion of the protocol, during which he or she was asked to mimic the laryngeal posture of laughter in the absence of generating a laugh while undergoing stroboscopic videolaryngoscopy.

Fiberscopic videolaryngoscopy was performed with the following equipment: an Olympus ENF-L3 flexible fiberscope, a Stryker 782 3-chip CCD camera, a Sony VO-9600 video recorder, and a Karl Storz 610 xenon light source. A Nagashima SFT-1 rigid telescope, an Elmo MN401 CCD camera, an Electrovoice RE-10 unidirectional microphone, and a Kay Rhino-Laryngoscope Stroboscope 9100 were used for the stroboscopic videolaryngoscopy (Fig 1). A Sony video printer UP-5000 was used to provide still images for study and publication.

At the conclusion of the videolaryngoscopy sessions, all video sequences were viewed at regular speed and in slow motion by all three authors.

RESULTS

All subjects tolerated the endoscopic procedure well and were able to complete all components of the protocol. All subjects reported that their simultaneous viewing of the videolaryngoscopy images as they were completing each task was very helpful. All video sequences were of excellent quality and provided information for study. Each author agreed with the final qualitative assessment of each video sequence.

During the first task (laughter on each vowel sound), the vocal folds underwent rhythmic abduction and adduction. The glottis modulated airflow by this valving maneuver to produce the characteristic vocalizations of laughter. These vocal fold movements occurred during laughter at each vowel sound, although supraglottic configuration and laryngeal elevation changed to produce the elemental vowel sound for each laughter type.

When each subject laughed in the different frequencies, similar results were observed. Again, the vocal folds were found to undergo their rhythmic valving movements of abduction and adduction. Pitch alterations were not achieved by changes in this rhythmic glottic valving, which appeared relatively constant; instead, each subject raised or lowered his or her entire larynx (in the superior-inferior direction) in order to control the pitch of the laughter.

In the third task, the larynx was observed during

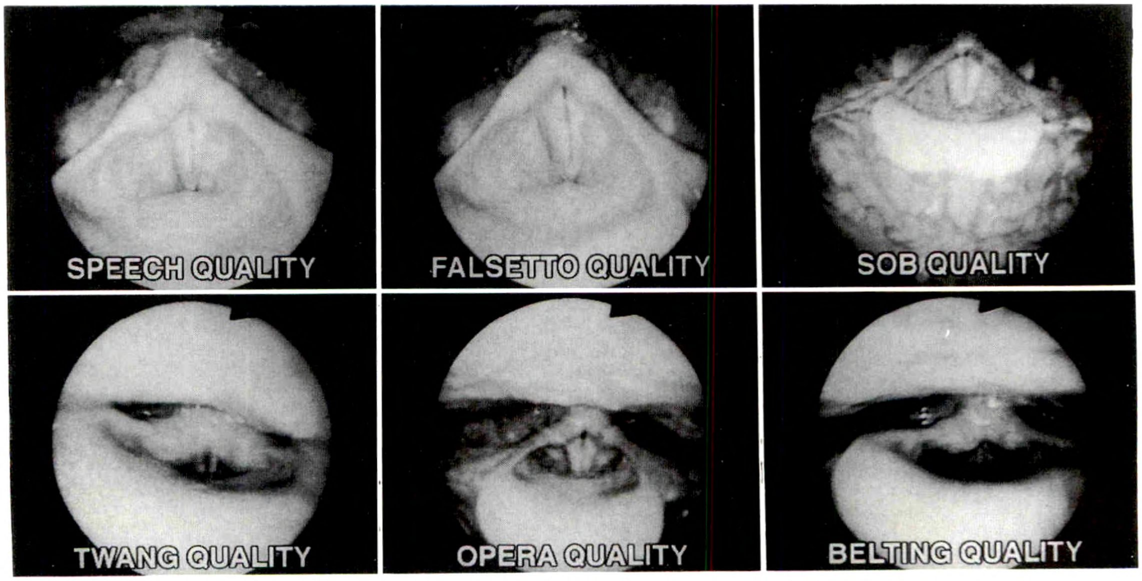

Fig 2. Representative still images of fiberscopic videolaryngoscopy of laughter in speech, falsetto, sob, twang, opera, and belting qualities.

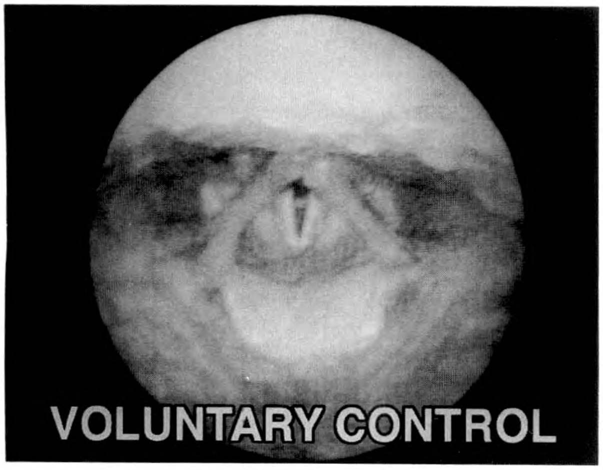

Fig 3. Paramedian true vocal fold positioning during gentle respiration.

laughter in the speech, falsetto, sob, twang, opera, and belting qualities. In all instances, the vocal folds underwent essentially identical rhythmic abduction and adduction, while the level of the larynx and the shape of the supraglottis were dramatically different for each voice quality (Fig 2).

At the conclusion of the three tasks, each subject attempted to "laugh silently" while viewing simultaneous videolaryngoscopy images of his or her larynx. All subjects were then able to voluntarily position the true vocal folds in both the abducted and adducted laugh postures (Fig 3). This exercise was then extended by having each subject attempt to intentionally place his or her true vocal folds in the paramedian position during gentle respiration. Each subject also successfully achieved this objective.

The subjects applied this skill of true vocal fold positioning and developed the ability to constrict and retract the supraglottis (Fig 4). During retraction, the supraglottis adopts an open configuration, in which more true vocal fold is visible from above, since the false vocal folds are at least partially lateralized. In contrast, during constriction, the false vocal folds are in a more medial position. All subjects were able to alternate between the retraction and constriction configurations during gentle phonation.

DISCUSSION

Laughter functions as a communication method that is easily recognized by members of all human societies, despite their differences in language, culture, and individual experiences.[6] The outward manifestations of laughter are relatively constant in all situations, but the stimuli and their interpretations may vary greatly.

Numerous observations of laughter have been made. The characteristic facial expression, including smiling and reflex lacrimation, has been well described.[7] Laughter also produces a generalized increase in skeletal muscle activity.[8] Activation of the autonomic nervous system leads to an initial increase, and then a decrease, in blood pressure and pulse.[6] These physiologic changes have been compared to physical exercise.[9]

All laughter is also characterized by similar vocalizations that involve both the upper aerodigestive tract and the respiratory system. The patterns of chest wall movement seen during laughter and speech are not identical,[10] and electromyographic recordings of abdominal muscles during laughter do not resemble those obtained during phonation.[11] In fact, specific respiratory patterns differentiate the emotional state of "joy-laughter" from other emotional states.[12]

Prior reports of the role of the larynx in laughter are limited. Observations of cricothyroid muscle and thyroarytenoid muscle contraction during laughter have been made.[13] Of note, an acoustic analysis of spontaneous laughter demonstrated signal noise and breathiness, suggesting a more abducted vocal fold positioning during laughter vocalization.[14] A Medline

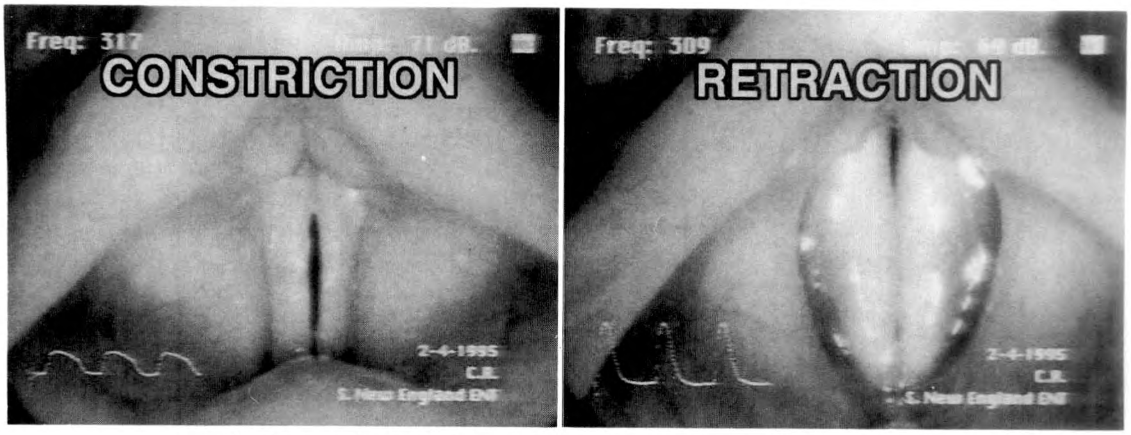

Fig 4. Supraglottic constriction and retraction during gentle phonation.

literature review did not reveal any published observations of laryngeal function during laughter.

In this study, videolaryngoscopy was performed on subjects during laughter. In all instances, characteristic vocal fold movements were observed: the vocal folds underwent rhythmic abduction and adduction. This "glottic valving" was present during laughter on all vowels, in all frequencies, and in each voice quality.

This observed posture of the larynx during laughter is unique. In other laryngeal functions, the sphincteric activity of the larynx predominates. In order to protect the larynx during swallowing, the true and false vocal folds are adducted and the epiglottis is displaced posteriorly over the laryngeal inlet. For the generation of subglottic pressures (ie, coughing), vocal fold adduction is essential. Phonation depends on true vocal fold adduction. In contrast, in laughter, abduction, as well as adduction, is present.

These observations have clinically important implications. After laughing in the different vowels, at various frequencies, and in several voice qualities, each subject developed the capability for voluntary paramedian vocal fold positioning. In particular, the subjects modulated false fold constriction and retraction. From this study, one cannot completely separate the effects of a subject's observation of his or her own larynx during phonation (ie, "biofeedback") from the effects of the specific laughter phonation tasks. Of note, each subject practiced the three laughter phonation tasks prior to videolaryngoscopy. The videolaryngoscopy served simply to record previously learned skills, and during simultaneous videolaryngoscopy, the subjects did little to modulate their laughter phonation maneuvers for each task. Furthermore, in a separate study (unpublished data), a second group of subjects completed a similar protocol without any simultaneous "biofeedback" videolaryngoscopy, and the vocal tract configuration during laughter was the same. Consequently, the "biofeedback" effects seem minimal, although they cannot be completely excluded.

Vocal fold hyperadduction contributes to dysphonias, such as vocal fold nodules and ventricular phonation. Patients with these conditions may be able to retrain their larynges to lessen the tendency for hyperadduction. In fact, at least one report states that laughter, by precipitating false vocal fold abduction, improved the voice quality of a patient with dysphonia plicae ventricularis.[15] Anecdotal reports also suggest a role for laughter in voice therapy (J. Estill, unpublished observations). These data also support the notion that laughter may be a potential component of voice therapy for hyperadduction dysphonias.

The precise mechanism for laughter's clinical effects in voice therapy is unknown. Possibly, laughter, a systemic somatic event, precipitates generalized vocal tract relaxation; however, this study suggests that the major vocal tract effects of laughter are at the level of the vocal folds. Furthermore, this study indicates that individuals may actively adopt the laryngeal posture of laughter (as seen in the second part of the protocol). Consequently, the beneficial effects of laughter for the treatment of specific dysphonias may be derived from voluntary vocal fold positioning, and not simple vocal tract relaxation. Clearly, additional research is necessary.

CONCLUSIONS

1. During laughter, both the false and true vocal folds undergo rhythmic abduction and adduction. This glottic valving was observed regardless of laughter frequency, principal vowel, or voice quality.

2. Laughter may be a useful component of voice therapy for hyperadduction dysphonias.

REFERENCES

1. Dorland's Illustrated medical dictionary. 27th ed. Philadelphia, Pa: WB Saunders, 1988.
2. Black DW. Laughter. JAMA 1984;252:2995-8.
3. Colton RH, Estill J. Elements of voice quality: perceptual, acoustic and physiological aspects. In: Lass NJ, ed. Speech and language: advances in basic research and practice. New York, NY: Academic Press, 1981:311-403.
4. Estill J. Belting and classic voice quality: some physiological differences. Med Problems Performing Artists 1988;3:37-43.
5. Yanagisawa E, Kmucha ST, Estill J, Leder SB. The contribution of aryepiglottic constriction to "ringing" voice quality. J Voice 1989;3:342-50.
6. Askenasy JJM. The functions and dysfunctions of laughter. J Gen Psychol 1987;114:317-34.
7. Pollio H, Mers R, Luchesi W. Humor, laughter and smiling. In: Goldstein J, ed. Psychology of humor. New York, NY: Academic Press, 1972:211-42.
8. Nirenberh SA. Normal and pathologic laughter in children. Clin Pediatr (Phila) 1991;30:630-2.
9. Bellert JL. Humor: A therapeutic approach in oncology nursing. Cancer Nurs 1989;12:65-70.
10. Bright K, Hixon T, Hoit J. Respiration as a laughing matter. Whimsy IV: humor across the disciplines, 1986;4:147-8.
11. Hoit JD, Plassman BL, Lansing RW, Hixon TJ. Abdominal muscle activity during speech production. J Appl Physiol 1988;65:2656-64.
12. Bloch S, Lemeignan M, Aguilera-T N. Specific respiratory patterns distinguish among human basic emotions. Int J Psychophysiol 1991;11:141-54.

13. Stearns FR. Laughing. Springfield, Ill: Charles C Thomas, 1972.

14. Bickley C, Hunnicutt S. Acoustic analysis of laughter. Proceedings of International Conference on Spoken Language 1992;2:927-30.

15. Brewer DW, McCall G. Visible laryngeal changes during voice therapy. Fiber-optic study. Ann Otol Rhinol Laryngol 1974;83:423-7.

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FUNCTIONAL ENDOSCOPIC SINUS SURGERY: BASIC AND ADVANCED TECHNIQUES

The course Functional Endoscopic Sinus Surgery: Basic and Advanced Techniques will be held in Graz, Austria. The English course will be held July 11-13, 1996, and the German course September 5-7, 1996. For further information, contact Prof H. Stammberger, MD, ENT Department, University Hospital, Auenbruggerplatz 20, A-8036 Graz, Austria; fax ++43 316 385 3425.

Ann Otol Rhinol Laryngol 105:1996

NITRERGIC INNERVATION OF THE RAT LARYNX MEASURED BY NITRIC OXIDE SYNTHASE IMMUNOHISTOCHEMISTRY AND NADPH-DIAPHORASE HISTOCHEMISTRY

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We evaluated the involvement of nitric oxide (NO) in the laryngeal innervation of rats using NADPH-diaphorase (NADPH-d) histochemistry and neuronal nitric oxide synthase (nNOS) immunohistochemistry. The findings obtained by NADPH-d histochemistry were identical with those obtained by nNOS immunohistochemistry, indicating that NADPH-d is nNOS in the laryngeal innervation system. We found NADPH-d–positive nerve fibers in every region of the larynx. In the epithelia of the mucosa, a small number of NADPH-d–positive nerve fibers were detected. The plexus of NADPH-d–positive nerve fibers was commonly found in the lamina propria, and some of these fibers were clearly associated with blood vessels. We also noted NADPH-d–positive nerve fibers in the region of laryngeal glands. Some of these fibers appeared to terminate in the glandular cells. We found NADPH-d–positive nerve fibers with varicosities in the intrinsic laryngeal muscle and free-ending nerve fibers on the muscle fiber. Motor end plate–like structures were positive for NADPH-d histochemistry. The NADPH-d–positive nerve fibers appeared to terminate at motor end plate–like structures in two of nine rats examined. A cluster of NADPH-d–positive neurons were occasionally present in the lamina propria of the laryngeal mucosa, in the connective tissue between the thyroid cartilage and intrinsic laryngeal muscle, and in the connective tissue near the cricoarytenoid joint. The present findings suggest that NO participates in the autonomic, sensory, and motor innervation of the larynx.

KEY WORDS — laryngeal innervation, NADPH-diaphorase, nitric oxide, nitric oxide synthase, rat.

INTRODUCTION

Until recently, nitric oxide (NO) was considered a toxic molecule, recognized as a destroyer of ozone, suspected carcinogen, and precursor of acid rain. It is now found to be essential to activities that range from digestion and blood pressure regulation to antimicrobial defense.[1] Nitric oxide also carries important information in the nervous system. It is considered to be a transmitter or signaling molecule in a number of distinct subpopulations of neurons in the central and peripheral nervous systems.[2] We have examined the catecholaminergic and peptidergic innervation of the larynx.[3-6] Many kinds of neuropeptides, as well as noradrenaline, have been clarified to participate in the autonomic, sensory, and motor innervation of the larynx. It is noteworthy to examine the participation of NO as a neuromodulator in the laryngeal innervation system.

Nitric oxide is not stored in neurons, but is synthesized by nitric oxide synthase (NOS). In the present study, we histochemically examined the localization of NO in the larynx of rats using NADPH-diaphorase (NADPH-d) histochemistry and neuronal NOS (nNOS) immunohistochemistry.

MATERIALS AND METHODS

Eleven Wistar rats of either sex were used. Under deep anesthesia induced by intraperitoneal administration of pentobarbital (30 mg/kg), the animals were perfused with 300 mL of 0.1 mol/L phosphate buffer (PB) with 2% paraformaldehyde, and the larynx was removed. The extirpated larynx was postfixed for 2 hours at 4°C in the same fixative, immersed overnight in 0.1 mol/L PB containing 20% sucrose, and sectioned on a cryostat (20 μm thick). Sections were collected on chrome-gelatine–coated glass slides for NADPH-d histochemistry. All sections were incubated for 60 minutes at 37°C with agitation in the following medium: 10 mg/mL nicotinamide adenine dinucleotide phosphate tetrasodium salt, reduced form (β-NADPH; Wako Pure Chemicals, Osaka, Japan), 0.5 mg/mL nitroblue tetrazolium (Merck, Darmstadt, Germany), 0.1% Triton X-100 in 0.1 mol/L phos-

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From the Departments of Otolaryngology (Hisa, Tadaki, Uno, Koike) and Anatomy (Tanaka, Ibata), Kyoto Prefectural University of Medicine, Kyoto, and the Department of Anatomy and Neurobiology, Kobe University School of Medicine, Kobe (Okamura), Japan. This work was supported by a Grant-in-Aid for Scientific Research (No. 08671978) from the Ministry of Education, Science and Culture, Japan.

Presented at the meeting of the American Broncho-Esophagological Association, Palm Desert, California, May 2-4, 1995.

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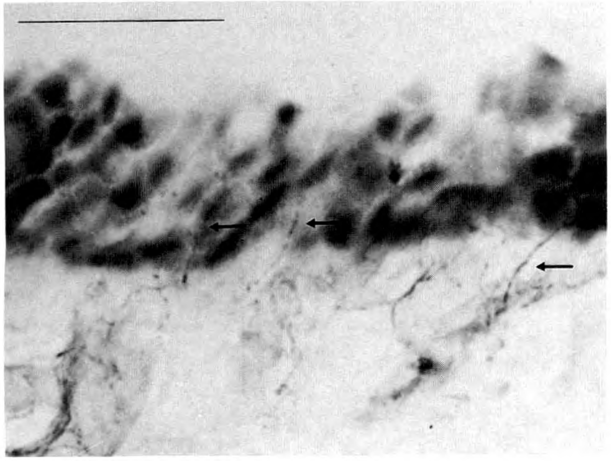

Fig 1. NADPH-d–positive nerve fiber with varicosities (arrows) in laryngeal epithelium. Bar — 50 μm.

phate-buffered saline (PBS), pH 7.4. The reaction was stopped by rinsing the sections in 0.1 mol/L PBS at 4°C.

In two rats, sections were prepared for nNOS immunohistochemistry before staining for NADPH-d activity. The mounted sections were incubated with rabbit anti-nNOS serum (1:1,000, Euro-diagnostica, Malmo, Sweden) for 48 hours at 4°C. The production and the specificity of the anti-nNOS serum was previously reported elsewhere.[7] The nNOS immunoreactivity was visualized by incubating sections with fluorescein-labeled anti-rabbit IgG (1:200, Vector Laboratories, Inc, Burlingame, Calif) for 1 hour at room temperature. After the sections were rinsed and photographed under a fluorescence microscope, they were processed for NADPH-d histochemistry as described above. The distribution of NADPH-d reactions under bright-field microscopy was compared with that of previously photographed nNOS immunoreactivity.

RESULTS

Nerve fibers positive for NADPH-d were found in every region of the larynx. In the epithelia of the laryngeal mucosa, a small number of NADPH-d–positive nerve fibers were detected in all rats examined. Most of these fibers with varicosities reached the surface of the epithelium and appeared to be free-ended (Fig 1). The plexus of NADPH-d–positive nerve fibers was frequently found in the lamina propria in all rats examined. Some of these fibers were clearly associated with blood vessels. This association had no relation to the size of the artery and vein. The distribution of NADPH-d–positive nerve fibers in the submucosa differed among regions of the larynx. These fibers were abundant in the supraglottis, especially the laryngeal surface of the epiglottis, and the subglottis, while relatively few fibers were seen in the glottis. We also found NADPH-d–positive nerve fibers in the region of the laryngeal glands in all rats examined. Some of these fibers appeared to terminate in glandular cells (Fig 2A).

We found NADPH-d–positive nerve fibers with varicosities in the thyroarytenoid muscle of all rats examined. Free ending–like fibers on the muscle fibers were noted (Fig 3A). Motor end plate–like structures were detected and NADPH-d–positive nerve fibers appeared to terminate on some motor end plate–like structures in two of the nine rats examined (Fig 3B). Abundant NADPH-d–positive nerve fibers were also associated with blood vessels in the muscle (Fig 3C).

A cluster of NADPH-d–positive neurons were occasionally found in the lamina propria of the laryngeal mucosa, in the connective tissue between the thyroid cartilage and intrinsic laryngeal muscle, and in the connective tissue near the cricoarytenoid joint. Many of these cells were strongly stained (Fig 4A).

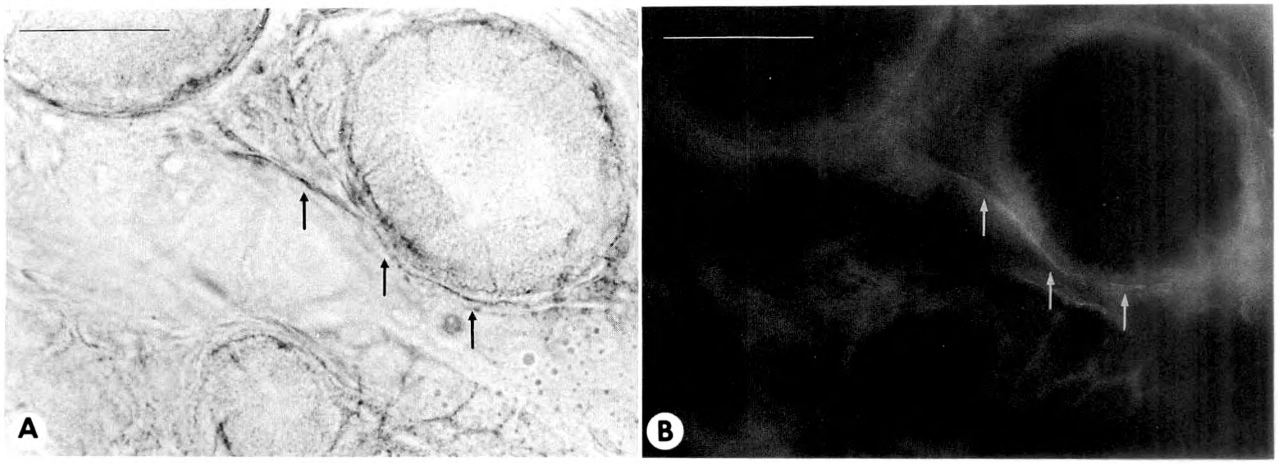

Fig 2. Double staining of A) NADPH-d histochemistry and B) neuronal nitric oxide synthase (nNOS) immunohistochemistry. Bars — 50 μm. A) NADPH-d–positive nerve fiber (arrows) surrounding acinar cell of submucosal gland. B) NADPH-d–positive nerve fiber in A is also nNOS-immunoreactive (arrows).

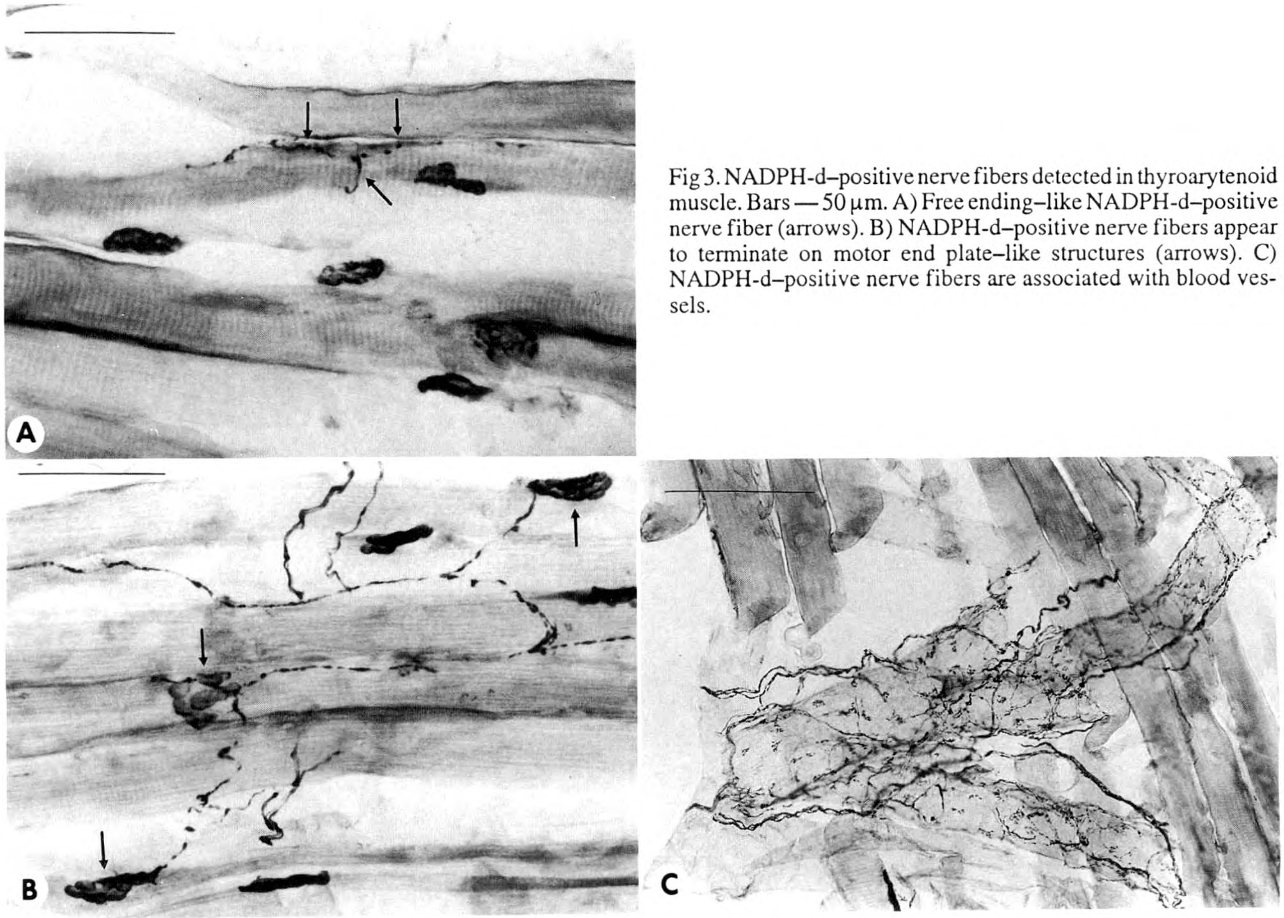

Fig 3. NADPH-d–positive nerve fibers detected in thyroarytenoid muscle. Bars — 50 μm. A) Free ending–like NADPH-d–positive nerve fiber (arrows). B) NADPH-d–positive nerve fibers appear to terminate on motor end plate–like structures (arrows). C) NADPH-d–positive nerve fibers are associated with blood vessels.

The findings obtained by NADPH-d histochemistry corresponded well with those obtained by nNOS immunohistochemistry on the same sections in the two rats examined (Figs 2 and 4).

DISCUSSION

It is now generally thought that NADPH-d is identical to nNOS.[8-10] We also reported that NADPH-d is identical to nNOS in the canine superior cervical ganglion (SCG).[11] The present study indicates that NADPH-d is nNOS also in the laryngeal innervation system, since the findings obtained by NADPH-d histochemistry were identical to those obtained by nNOS immunohistochemistry.

In the autonomic innervation, the participation of NO differs among species. We found a considerable number of NADPH-d–positive neurons in the canine SCG.[11] In contrast, neurons in the rat SCG were not

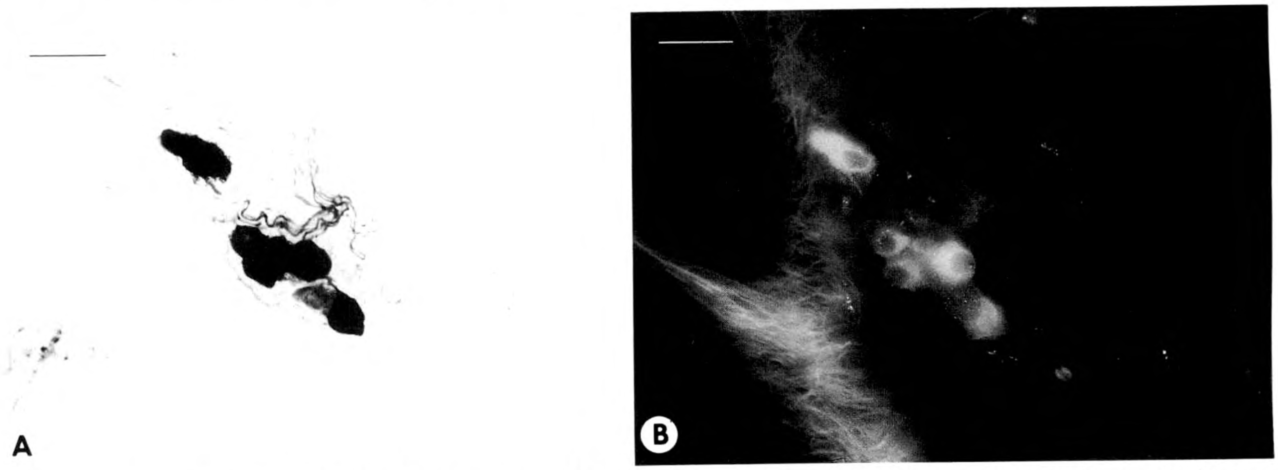

Fig 4. Double staining of A) NADPH-d histochemistry and B) nNOS immunohistochemistry. Bars — 50 μm. A) Cluster of stained cells in lamina propria of supraglottic mucosa. B) NADPH-d–positive cells in A are also nNOS-immunoreactive.

positive for NADPH-d, but they were enveloped by a mesh of intensely staining fibers.[10] This finding rules out the possibility of NO involvement in the sympathetic innervation of the rat larynx, since the main source of sympathetic nerve fibers in the larynx is the SCG.[3]

Nitric oxide has been considered as an endogenous modulator of cholinergic neurotransmission in the guinea pig airway.[12] The present study demonstrated the presence of a cluster of densely stained cells in the larynx of the rat. The intralaryngeal ganglia of cats were studied in detail by Yoshida et al.[13] They found ganglia containing 50 to 80 ganglionic neurons per ganglion mainly in the paraglottic space, and suggested that these neurons have an endogenic autonomic, especially cholinergic, nature and innervate vessels and glands. The cells in the intralaryngeal ganglia of rats have been reported to be all acetylcholinesterase-positive; this finding suggests that these ganglia are parasympathetic ganglia.[14] These studies suggest that the cluster of NADPH-d–positive neurons found in the present study represents parasympathetic ganglia in the larynx, and that NO is also an endogenous modulator of cholinergic neurotransmission in the larynx of the rat. This idea is also supported by the present findings that blood vessels and submucosal glands in the larynx are supplied with NADPH-d–positive nerve fibers. The present study suggests that NO is an endogenous modulator of cholinergic neurotransmission in the parasympathetic innervation for the regulation of blood flow and secretion in the larynx.

Participation of NO in the parasympathetic ganglia has been examined in the lower airway,[15] gall bladder,[16] and pelvic organs.[17] Nitric oxide is the neuromodulator of inhibitory nonadrenergic noncholinergic (NANC) nerve fibers for the smooth muscle in these organs. Both sympathetic and vagal sensory ganglia are sources of inhibitory NANC nerve fibers in the lower airway, in contrast to postganglionic neurons in the gallbladder and pelvic organs. There seems to be no participation of NANC nerve fibers in the principal functions of the larynx, since no smooth muscle except for the blood vessels exists there. The possibility of inhibitory NANC innervation of NO originating from vagal sensory ganglia also exists. The sensory innervation to the larynx is supplied by the nodose ganglion.[6] Many neurons in this ganglion have been demonstrated to be nitrergic.[18,19]

In the periphery, NO has been suggested to activate primary afferents that modulate nociceptive behavior and to mediate analgesia when hyperalgesia has been induced by prostaglandin E2.[20-22] We found many substance P (SP) nerve fibers and calcitonin gene–related peptide (CGRP) nerve fibers with varicosities, possibly afferent nerve fibers, in the epithelium of the canine laryngeal mucosa.[4,5] In the present study, we noted only a small number of such afferent nerve fibers positive for NADPH-d in the epithelium of the laryngeal mucosa. However, this finding cannot exclude the existence of a considerable number of afferent NADPH-d–positive nerve fibers, since the technical limitation that the epithelial cells were strongly stained possibly prevents the precise detection of the positive nerve fibers. Moreover, a plexus of NADPH-d–positive nerve fibers, apart from the blood vessels and glands, was frequently found in the lamina propria in the laryngeal mucosa. The present finding of free ending–like fibers on the muscle fiber in the thyroarytenoid muscle suggests that the nociception of this intrinsic laryngeal muscle is also mediated by NO.

Generally, nNOS is undetectable in motoneurons of the brain stem and spinal cord. However, it has been recently demonstrated to be localized in motoneurons of cranial nerves and spinal cord under some conditions.[23,24] However, there is a fundamental difference in the regulation of nNOS expression between motoneurons of the cranial and spinal nerves. Wu[23] reported that spinal motoneurons express the enzyme after ventral root avulsion, but not after ventral transection, and that early nNOS expression is associated with axonal sprouting and growth and the neurons expressing lesion-induced nNOS ultimately die. The present finding, that NADPH-d–positive nerve fibers appeared to terminate on some of the motor end plate–like structures of the thyroarytenoid muscle in two of nine rats examined, might be related to axonal sprouting, since fiber type grouping in the intrinsic laryngeal muscle is well known.[25] This remains to be clarified in view of the NO participation in the peripheral motor innervation system.

The present findings suggest that NO participates in the autonomic, sensory, and motor innervation of the larynx.

REFERENCES

1. Culotta E, Koshland DE Jr. NO news is good news. Science 1992;258:1862-5.

2. Snyder SH. Nitric oxide: first in a new class of neurotransmitter? Science 1992;257:494-6.

3. Hisa Y. Fluorescence histochemical studies on the noradrenergic innervation of the canine larynx. Acta Anat (Basel) 1982;113:15-25.

4. Hisa Y, Sato F, Fukui K, Ibata Y, Mizukoshi O. Substance

P nerve fibers in the canine larynx by PAP immunohistochemistry. Acta Otolaryngol (Stockh) 1985;100:128-33.

5. Hisa Y, Uno T, Murakami Y, Okamura H, Ibata Y. Distribution of calcitonin gene–related peptide nerve fibers in the canine larynx. Eur Arch Otorhinolaryngol 1992;249:52-5.

6. Hisa Y, Tadaki N, Uno T, Okamura H, Taguchi J, Ibata Y. Neuropeptide participation in canine laryngeal sensory innervation. Immunohistochemistry and retrograde labeling. Ann Otol Rhinol Laryngol 1994;103:767-70.

7. Alm P, Larsson B, Ekblad E, Sundler F, Andersson K-E. Immunohistochemical localization of peripheral nitric oxide synthase–containing nerves using antibodies raised against synthesized C- and N-terminal fragments of a cloned enzyme from rat brain. Acta Physiol Scand 1993;148:421-9.

8. Hope BT, Michael GJ, Knigge KM, Vincent SR. Neuronal NADPH diaphorase is a nitric oxide synthase. Proc Natl Acad Sci USA 1991;88:2811-4.

9. Grozdanovic Z, Baumgarten HG, Brüning G. Histochemistry of NADPH-diaphorase, a marker for neuronal nitric oxide synthase, in the peripheral autonomic nervous system of the mouse. Neuroscience 1992;48:225-35.

10. Morris R, Southam E, Gittins SR, Garthwaite J. NADPH-diaphorase staining in autonomic and somatic cranial ganglia of the rat. NeuroReport 1993;4:62-4.

11. Hisa Y, Uno T, Tadaki N, Umehara K, Okamura H, Ibata Y. NADPH-diaphorase and nitric oxide synthase in the canine superior cervical ganglion. Cell Tissue Res 1995;279:629-31.

12. Belvisi MG, Stretton D, Barnes PJ. Nitric oxide as an endogenous modulator of cholinergic neurotransmission in guinea pig airways. Eur J Pharmacol 1991;198:219-21.

13. Yoshida Y, Shimazaki T, Tanaka Y, Hirano M. Ganglions and ganglionic neurons in the cat's larynx. Acta Otolaryngol (Stockh) 1993;113:415-20.

14. Domeij S, Dalqvist A, Forsgren S. Enkephalin-like immunoreactivity in ganglionic cells in the larynx and superior cervical ganglion of the rat. Regul Pept 1991;32:95-107.

15. Fischer A, Mundel P, Mayer B, Preissler U, Philippin B, Kummer W. Nitric oxide synthase in guinea pig lower airway innervation. Neurosci Lett 1993;149:157-60.

16. Talmage EK, Mawe GM. NADPH-diaphorase and VIP are co-localized in neurons of gallbladder ganglia. J Auton Nerv Syst 1993;43:83-90.

17. Vizzard MA, Erdman SL, deGroat WC. Localization of NADPH-diaphorase in pelvic afferent and efferent pathways of the rat. Neurosci Lett 1993;152:72-6.

18. Aimi Y, Fujimura M, Vincent SR, Kimura H. Localization of NADPH-diaphorase-containing neurons in sensory ganglia of the rat. J Comp Neurol 1991;306:382-92.

19. Morris R, Southam E, Gittins SR, Garthwaite J. NADPH-diaphorase staining in autonomic and somatic cranial ganglia of the rat. Neuroreport 1993;4:62-4.

20. Duarte IDG, Lorenzetti BB, Ferreira SH. Peripheral analgesia and activation of the nitric oxide–cyclic GMP pathway. Eur J Pharmacol 1990;186:289-93.

21. Meller ST, Lewis SJ, Bates JN, Brody MJ, Gebhart GF. Is there a role for an endothelium-derived relaxing factor in nociception? Brain Res 1990;531:342-5.

22. Morris R, Southam E, Braid DJ, Garthwaite J. Nitric oxide may act as a messenger between dorsal root ganglion neurones and their satellite cells. Neurosci Lett 1992;137:29-32.

23. Wu W. Expression of nitric-oxide synthase (NOS) in injured CNS neurons as shown by NADPH diaphorase histochemistry. Exp Neurol 1993;120:153-9.

24. Clowry GJ. Axotomy induces NADPH diaphorase activity in neonate but not adult motoneurones. NeuroReport 1993;5:361-4.

25. Malmgren LT, Gacek RR. Histochemical characteristics of muscle fiber types in the posterior cricoarytenoid muscle. Ann Otol Rhinol Laryngol 1981;90:423-9.

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DIZZINESS AND BALANCE DISORDERS

The Eighth Annual Interdisciplinary Seminar on Diagnostic and Rehabilitative Aspects of Dizziness and Balance Disorders will be held December 4-7, 1996, in Denver, Colorado. For further information, contact Jane Wells, MA, 1996 Balance Meeting, 300 E Hampden Ave, Suite 401, Englewood, CO 80110; telephone (303) 788-4235; fax (303) 788-4234.

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PEDIATRIC TRACHEOBRONCHIAL FOREIGN BODIES: HISTORICAL REVIEW FROM THE JOHNS HOPKINS HOSPITAL

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We investigated changing trends in pediatric tracheobronchial foreign body removal and resident experience from 1939 to 1991. We retrieved the records of 234 cases of tracheobronchial foreign body removal at Johns Hopkins. The mean number of cases per year was 5.9. The most common foreign bodies removed were peanuts, accounting for 38.9%. The average yearly incidence of pediatric tracheobronchial foreign bodies remained relatively constant during the period studied. Our data suggested little change in outcome or complications with the advent of optical telescopes in the mid-1970s, despite their great value in improved visualization. Resident experience and training were evaluated by the number of cases attended by each resident during his or her training. The number varied from 1 to 8 cases, not including experience acquired at our sister institutions. Although complete data could not be obtained in many of the older medical records, our review suggests that despite the advantage offered by the optical forceps technology, proper training and experience in traditional rigid endoscopic techniques is still crucial to optimize outcome and minimize the risk of complications in pediatric tracheobronchial foreign body removal. Chevalier Jackson's recommendation that residency training include an animal laboratory course in foreign body removal still applies.

KEY WORDS — aspiration, endoscopy, foreign body.

INTRODUCTION

Foreign body aspiration is a serious and potentially fatal occurrence in childhood. A cause of worldwide morbidity and mortality, it most commonly occurs in children 6 months to 4 years of age, with 2,000 to 3,000 cases reported annually in the United States.[1-3] We documented 234 cases of pediatric airway foreign bodies at The Johns Hopkins Hospital from 1939 to 1991 and reviewed resident training and experience over this period. The following discussion compares our data to those of previous reports and presents cases that illustrate similarities and differences in foreign body practice over the past 50 years. We believe this review supports the thesis that clinical experience alone is seldom adequate to prepare the resident for the variety and complexity of foreign body cases that may be encountered in future practice.

Historically, the first foreign body removal by bronchoscopic technique was performed in 1897 by Gustav Killian. This was preceded by the first direct laryngoscopy in 1895 by Kirstein, and the first bronchoscopy later that same year by Killian. Killian's students William Bruenings and Carl von Eiken helped further develop Killian's bronchoscope.[4] Collidge performed the first successful bronchoscopy in the United States in 1898, when he extracted a portion of a tracheal cannula from the bronchus of a 23-year-old man. Max Einhorn introduced a light carrier to the esophagoscope in 1902. Prior to this time, all illumination was from external sources.[5] The technique and instrumentation were further modified and expanded in the United States by Chevalier Jackson. Jackson is credited with developing the basic diagnostic and therapeutic principles used in evaluating foreign body aspiration and also with advancing pediatric foreign body aspiration as a public health concern. Edwin Broyles introduced magnification in the 1940s and the fiberoptic illuminator in 1962. Paul Bunker in the 1960s directed a nationwide educational program sponsored by the American Broncho-Esophagological Association to provide education and information regarding all foreign body accidents.[6]

The mortality rate prior to the introduction of bronchoscopic techniques promulgated by Chevalier Jackson was 50%. Current mortality rates with improved instrumentation and anesthetic techniques are less than 1%.[7,8]

The techniques reported by Jackson[9] in 1905, Jackson and Jackson[10] in 1936, and Holinger and Johnston[11] in 1954 are just as applicable today as when first described. Even with improved optics and anesthesia, "The fundamental problem is to teach the pupil how to put a rigid tube into the tender passages

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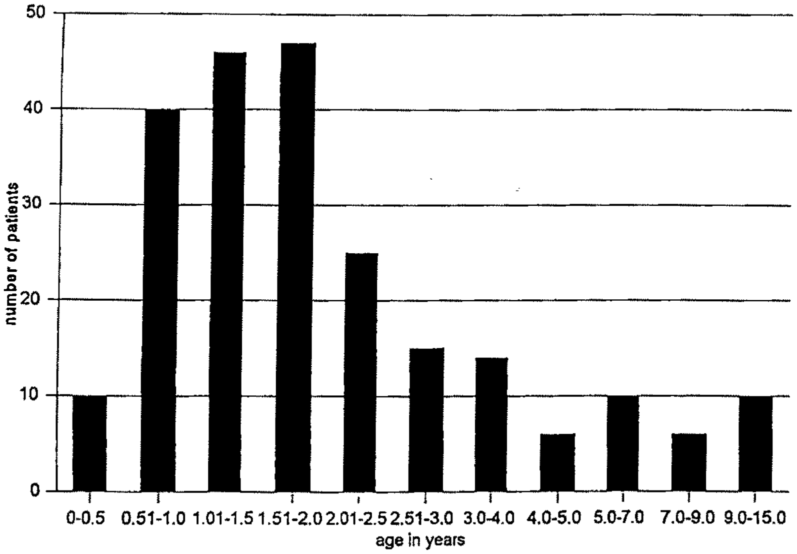


Fig 1. Age distribution of patients.

of an infant and to carry out certain complicated, potentially fatal manipulations with safety to the patient."[12(p3)]

MATERIAL AND METHOD

Tracheobronchial foreign body removal case records were obtained by two means. Cases from 1939 to 1963 were selected by a review of the general operating room logs. For the years 1963 to 1991, a computer-generated list was obtained by CPT (Current Procedural Terminology) code. Charts were then reviewed for pertinent data. The search was limited to patients less than 16 years of age, and 234 such cases were retrieved. From 1939 to 1963, many foreign bodies were removed in the endoscopic clinic for which records could not be located. No operating room logs were available for 1946 to 1952. Discussions with attending physicians and residents practicing before 1963 and review of the Johns Hopkins Hospital Foreign Body Collection suggest that our retrieval from these early years grossly underestimates the total clinical experience. The total number of cases during this time may be double what our data suggest. These data represent experience obtained at The Johns Hopkins Hospital and do not include experience gained at our sister institutions.

RESULTS

In our review of 234 cases, the age of the patient was available for 229. The age distribution ranged from 2 weeks to 15 years, with 78.7% of tracheobronchial foreign bodies occurring in children less than 3 years of age (Fig 1). The average age of children in the study was 2.1 years. The sex of 209 patients was available: 63.2% were male and 36.8% were female, with a male to female ratio of 1.7 to 1. The total number of documented cases per year at The Johns Hopkins Hospital varied, up to as many as 17, with a mean of 5.9. There was no statistically significant difference in the number of cases performed per year.

The type of foreign body was identified in 167 cases. Sixty percent of these foreign bodies were of vegetable origin. Peanuts accounted for 38.9% overall. An itemization of all foreign bodies retrieved (Table 1) and a photograph of representative items from the Johns Hopkins Hospital Foreign Body Collection (Fig 2) are included.

The locations of 196 foreign bodies were identified in 177 patients (Fig 3). A single foreign body was found in 161 patients. Multiple foreign bodies were found in 16 (9%) of the 177 patients (Table 2). Twenty-seven percent of foreign bodies were located in the right main stem bronchus, with 23% located in the right segmental bronchi, for a total of 50% right-sided foreign bodies. Fifteen percent were located in the left main stem bronchus, with 17% located in the left segmental bronchi, for a total of 32% left-sided foreign bodies. Ten of the 196 foreign bodies were listed as bronchus-only without specifying side, 23 were located in the trachea, and 3 were specified as laryngeal.

The time from the onset of symptoms to the surgery performed was available in 83 cases (Fig 4). Six patients (7%) were taken to the operating room within 6 hours, 34 patients (41%) within 6 to 24 hours, 16 patients (19%) within 24 to 72 hours, and 27 patients (33%) at greater than 72 hours.

In order to evaluate the training experience of our residents in airway foreign body removal, we identified the attending surgeon and the assistant for each case. The number of cases that each resident attended during training ranged from 1 to 8. These numbers do not reflect diagnostic bronchoscopy, esophageal for-

TABLE 1. FOREIGN BODIES (N = 167)

Type	*No.*	*%*
Peanut	65	38.9%
Plastic toy	14	8.4%
Safety pin, pin, or tack	13	7.8%
Seeds (watermelon and sunflower)	10	4.2%
Nail, screw, or nut	7	4.2%
Carrot	6	3.6%
Bacon	5	3.0%
Bone	5	3.0%
Popcorn	5	3.0%
Tooth	4	2.4%
Corn	3	1.8%
Bead	3	1.8%
Eggshell	3	1.8%
Apple	3	1.8%
Vegetable matter (unspecified)	2	1.2%
Spring	2	1.2%
Crayon	2	1.2%
Crab claw	2	1.2%
Candy	1	0.6%
Tracheal granuloma	1	0.6%
Ceramic	1	0.6%
Feeding tube	1	0.6%
Paper	1	0.6%
Grass	1	0.6%
Pine needle	1	0.6%
Polyp	1	0.6%
Medicine tablet	1	0.6%
Cotton	1	0.6%
Taco shell	1	0.6%
Cauliflower	1	0.6%
Stone	1	0.6%

eign body removal, or experience gained at sister institutions.

CASE REPORTS

Case 1. On December 3, 1941, a small child arrived at the Johns Hopkins Hospital emergency room with a history of having choked on a kernel of corn the day before. Physical examination revealed paroxysms of coughing and, according to the report, "Occasionally one could hear the foreign body against the lower surface of the vocal cords." Direct laryngoscopy revealed nothing definite, but it was believed that there was a foreign body moving up and down in the trachea. Edwin Broyles was immediately notified and the child was taken to the operating room, where a tracheotomy was performed under local anesthesia. A short No. 4 bronchoscope was passed through the tracheotomy and a large, swollen, yellow kernel of field corn was removed. A short No. 2 tracheotomy tube was placed and the patient was sent to the ward to be decannulated the following day. She was discharged in good condition on December 8, 1941. At the time this procedure was performed, Dr Broyles was still adamant that no child be submitted to bronchoscopy without a tracheotomy. This policy finally gave way during the subsequent year or two. Doubtless, this approach provides the advantage of a somewhat larger bronchoscope than could otherwise be used, in addition to a shorter instrument, facilitating foreign body removal. This approach may still be indicated in special circumstances.[13]

Case 2. On April 16, 1943, the 3-year-old daughter of a British naval officer in Bermuda experienced a sudden cough-choke episode at about 9:00 AM. A local physician found evidence of a bronchial obstruction and performed a direct laryngoscopy, failing to discover any foreign body. In the absence of any bronchoscopic equipment in Bermuda, she was placed on the *Bermuda Clipper*, arriving at Johns Hopkins Hospital later the same day. Examination revealed an audible wheeze and a click with diminished breath sounds over the left chest. A No. 5 bronchoscope was passed under ether anesthesia, demonstrating a bead-like object tightly impacted in the left lower lobe. Attempts to remove the object were unsuccessful because of lack of the proper bronchoscopic forceps. After 40 minutes the procedure was discontinued. The Pilling Company was called and an appropriate bronchoscope and forceps were sent by train, allowing for successful foreign body removal on April 17, 1943, by means of a "Tucker bead forceps."

Case 3. On January 5, 1984, a 7-month-old infant was brought to The Johns Hopkins Hospital with a history of choking on peanut brittle. The child's parent was unsure as to the length of time since the episode of aspiration. Physical and radiographic findings suggested a foreign body in the left lung. At bronchoscopy, the left main stem bronchus was almost occluded by edema and pus at the level of the left upper lobe. Careful suctioning revealed a small portion of nut nearly obscured by reactive changes. The swollen bronchus was too small to accommodate the fenestrated optical forceps. Therefore, it was necessary to use other instrumentation, namely, a thin blade, forward-grasping forceps and Pilling bronchoscope, to successfully retrieve the peanut fragment.

DISCUSSION

The classic diagnostic triad of sudden onset of paroxysmal coughing, wheezing, and diminished breath sounds on the affected side is not present in all cases of foreign body aspiration.[14] Chest radiographs can be normal in as many as one third of the cases.[15] Therefore, a high index of suspicion must be main-

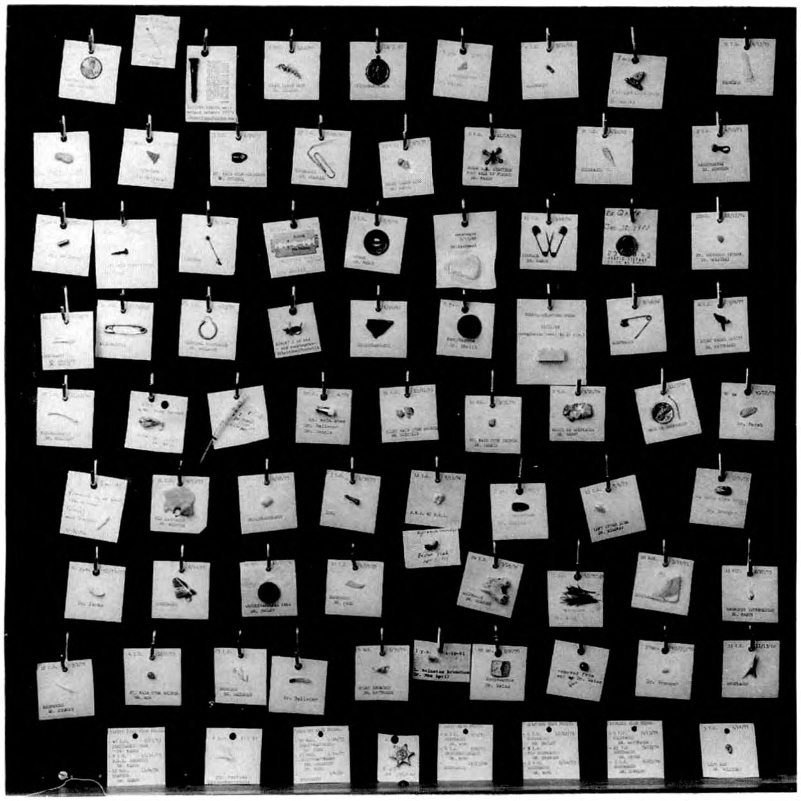


Fig 2. Johns Hopkins Hospital Foreign Body Collection.

tained, and bronchoscopy performed to rule out a foreign body, when clinically suspected. In most societies, vegetable matter is the most commonly reported inhaled foreign body, with nuts and seeds predominating.[14,16,17] Our data concerning age, sex, location and type of foreign body identified are consistent with those of previously published reports.[18-21] The frequency of significant complications in these patients did not change appreciably over the period studied, despite the advent of newer technologies, including the optical forceps. This seems inconsistent with a previous report suggesting a decreased incidence of complications with optical forceps.[22] This discrepancy may be a matter of definition as to what constitutes a complication. We do not mean by this statement that optical forceps are of little use. To the contrary, in many cases optical forceps and video technology greatly increase the ease of extraction and provide an excellent teaching tool that can benefit residents not directly involved in the case. However, this "ease" can create a false sense of security. These instruments are often inadequate for children under 1 year of age, for foreign bodies located in the smaller segmental bronchi, and for some cases in which a foreign body reaction has occurred, as is illustrated in case 3. In these circumstances, a traditional approach may be required.

# placement		%
Laryngeal	3	1.5
Tracheal	23	12.0
RMS	53	27.0
LMS	29	14.9
R segmented	45	23.0
L segmented	32	16.4
* Bronchus	10	

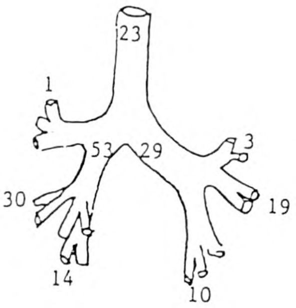


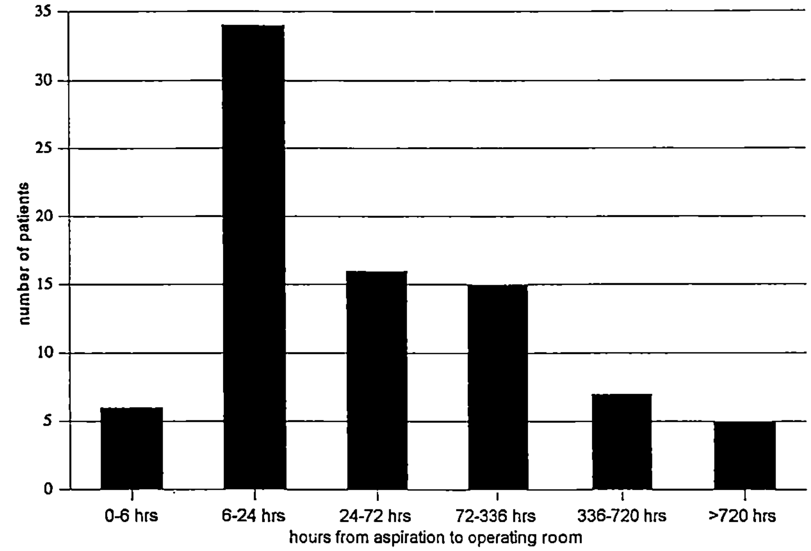
Fig 3. Location of 195 foreign bodies identified in 177 patients. Asterisk — 10 locations were identified as bronchus only, without specifying side, RMS — right main stem, LMS — left main stem.

Our review of resident experience and training highlights some important considerations in the prepa-

TABLE 2. MULTIPLE FOREIGN BODY LOCATIONS (N = 16)

	No. of Pts
Trachea, R main stem bronchus	3
Trachea, R middle lobe, L main bronchus	1
Trachea, R middle lobe	1
R main bronchus, R middle lobe	3
R middle lobe, R lower lobe	1
R middle lobe, L main stem bronchus	1
L main stem bronchus, L lower lobe	3
R middle lobe, L lower lobe	1
L upper lobe, 2 in L lower lobe	1
2 in R middle lobe	1

Fig 4. Time to surgery.



ration of residents to perform foreign body procedures after graduation. First, the types of foreign bodies encountered are numerous and require broad training in the use of specific instruments, as is illustrated in case 2. Both the technique and instruments used are dependent on the size, shape, and consistency of the specific foreign body. Over 33% of foreign bodies were removed later than 72 hours after aspiration. This delay increases the likelihood of a foreign body reaction, which increases the complexity of the case and consequently increases the risk of complications or failure. These considerations make it important that residents acquire extensive training in performing foreign body procedures before undertaking difficult cases in their individual careers.

We found considerable variation among individual residents in the number of cases attended. Even though these numbers underestimate the total clinical experience, this experience is far too limited to stand alone in preparing the resident for future pursuits in pediatric bronchoscopic foreign body removal. Even the residents who attended the most cases were seldom exposed to all of the special techniques needed or the unique instruments required to remove certain foreign bodies, such as safety pins, beads, ball bearings, and others. Because of this deficiency, the Johns Hopkins Hospital program has for more than 40 years supplemented resident training with a bronchoscopic foreign body course in the animal laboratory.

Our laboratory course is designed to train junior residents, while allowing more senior residents the opportunity to update their skills. All sessions are supervised by the senior author (B.R.M.), who has wide experience in bronchoscopic foreign body removal. The course is given in eight 2½-hour sessions. In the first four sessions dogs are used to simulate the airway of a larger child, while in the later four sessions cats are used to simulate the infant airway. Two anesthetized, spontaneously breathing animals are available for each session, and a full set of traditional bronchoscopes is available. We provide a complete set of foreign body forceps, including Jackson fenestrated forceps; bead forceps; Tucker tack and pin forceps; screw nail forceps; side curved forceps; double action, forward grasping, fenestrated forceps; etc. Optical forceps and telescopes are avoided because of cost and the necessity to prepare the residents for more difficult cases in the younger child, in whom such instruments may prove inadequate. A variety of foreign bodies is used, including peanuts, nails, screws, beads, pins, thumbtacks, and others. Each resident is given an opportunity to practice the removal of these different foreign bodies using the specialized forceps available. In addition, a human tracheobronchial tree model is used to familiarize the residents with the anatomy of the human airways. Removal of foreign bodies using flexible bronchoscopic technique is also practiced.

Resident training in bronchoscopic foreign body removal techniques has not received adequate emphasis from the modern otolaryngology community. In 1988, 52% of the 105 otolaryngology–head and neck surgery residency training programs in the United States had no animal laboratory to teach foreign body removal techniques, and of those that did, hands-on instruction was limited to 1 to 10 hours per resident in 75% of the responses. Nevertheless, 79% of the directors felt that their training programs adequately prepared their residents for pediatric bronchoscopic foreign body removal.[23]

With an increased number of otolaryngology–head and neck surgery residents as compared to earlier times, the number of foreign body cases managed per resident has declined. The endoscopic experience has also been diluted among the various specialties. Pediatric pulmonologists, gastroenterologists, and thoracic and pediatric surgeons have all increased their activity in this aspect of care, thus limiting the pool of patients necessary to train our residents. We were pleased to find that our department continues to play a dominant role in pediatric airway foreign body removal, even though other disciplines have become more involved in the management of esophageal foreign bodies.

It has been suggested that not every otolaryngology–head and neck surgery resident needs to be trained in tracheobronchial foreign body removal. Advocates of this view believe that the limited number of cases available should be funneled to residents planning a career in pediatric airway management, while others propose that further training might be obtained by means of a fellowship. In either case, the clinical material alone is seldom sufficient to adequately prepare the resident or fellow for a practice involving a broad range of foreign body work. We realize that these skills, once acquired through proper training, must be maintained through an active practice caring for pediatric airway disorders and repeated animal laboratory experience. We also know that purchasing and maintaining the array of instruments required is extremely costly. These points support the need for referral centers in which more difficult cases can be managed. However, emergency foreign body removal may be necessary outside of these centers, and the otolaryngologist will be expected to manage these cases in most instances. We feel strongly that the foreign body animal laboratory is essential to train residents in these techniques and should be a part of the core curriculum.

CONCLUSION

Tracheobronchial foreign body aspiration is a serious and potentially fatal situation, usually occurring in a small child or infant. The skill and dexterity needed to safely remove foreign bodies cannot be obtained with the number of operative cases residents attend during their training programs alone. In Dr Jackson's own words, training "certainly cannot be obtained to an adequate degree in a lifetime of clinical bronchoscopic work...No one should attempt the removal of a foreign body from the bronchus of a child until he has removed at least 100 foreign bodies from the dog without harming the animal." Additionally, "Endoscopic ability cannot be bought with instruments. As with all mechanical procedures, facility can be obtained only by educating the eye and the fingers in repeated exercise of a particular series of maneuvers."[24(p117)]

Newer optical instruments simplify the management of many cases, but such equipment is not yet available in sufficient variety to permit total reliance on this approach alone. This is especially true in children under 1 year of age and when foreign bodies are located in small distal bronchi. To prepare the resident for the broad range of cases likely to be encountered, a strong emphasis must be placed on training. We must dispel the notion that optical forceps and new technology make it unnecessary to fully train our residents with traditional instruments of proven efficacy over many years. We are repeatedly informed of misadventures and even fatalities resulting from lack of proper training in the use of traditional techniques. It is cause for concern that we may be training a generation of endoscopists who lack the skill of our predecessors and who are less competent in spite of improved technology and equipment. What a bronchoscopist needs is "gentleness, an exquisite sense of touch, caution, orientation, mechanical ability and patience. These can be acquired in a very remarkable degree by practice."[25(p206)]

REFERENCES

1. Blumhagen JD, Wesenberg RL, Brooks JG, Cotton EK. Endotracheal foreign bodies. Clin Pediatr (Phila) 1980;19:480-4.
2. Mofenson HC, Greensher J. Management of the choking child. Pediatr Clin North Am 1985;32:183-92.
3. Vane DW, Pritchard J, Colville CW, West KW, Eigen H, Grosfeld JL. Bronchoscopy for aspirated foreign bodies in children. Arch Surg 1988;123:885-8.
4. Fleischer K. Erkennung und Entfernung von Bronchialfremdkorpern — einst und jetzt. Ther Gegenw 1974;113:348-58.
5. Clerf LH. Historical aspects of foreign bodies in the air and food passages. Ann Otol Rhinol Laryngol 1952;61:5-17.
6. Friedberg SA, Bluestone CD. Foreign body accidents involving the air and food passages in children. Otolaryngol Clin North Am 1970;3:395-403.
7. Aytac A, Yurdakul Y, Ikizler C, Olga R, Saylam A. Inhalation of foreign bodies in children. J Thorac Cardiovasc Surg 1977;74:145-51.
8. Cohen SR, Herbert WI, Lewis GB Jr, Geller KA. Foreign bodies in the airway. Five-year retrospective study with special reference to management. Ann Otol Rhinol Laryngol 1980;89:437-42.
9. Jackson C. Foreign bodies in the trachea, bronchi and esophagus — the aid of esophagoscopy, bronchoscopy, and magnetism in their extraction. Laryngoscope 1905;15:257-81.
10. Jackson C, Jackson CL. Diseases of the air and food passages of foreign body origin. Philadelphia, Pa: WB Saunders, 1936.
11. Holinger PH, Johnston KC. Symposium on accidents and emergencies; foreign bodies in air and food passages. Pediatr Clin North Am 1954;1:827-43.
12. Jackson C. Teaching bronchoscopy and esophagoscopy. Arch Otolaryngol 1928;7:1-12.

13. Marks SC, Marsh BR, Dudgeon DL. Indications for open surgical removal of airway foreign bodies. Ann Otol Rhinol Laryngol 1993;102:690-4.

14. Linegar AG, Von Oppell UO, Hegemann S, De Groot M, Odell JA. Tracheobronchial foreign bodies. S Afr Med J 1992;82: 164-7.

15. Mu L, Sun D, He P. Radiological diagnosis of aspirated foreign bodies in children: review of 343 cases. J Laryngol Otol 1990;104:778-82.

16. Elhassani NB. Tracheobronchial foreign bodies in the Middle East. J Thorac Cardiovasc Surg 1988;96:621-5.

17. Abdulmajid OA, Ebeid AM, Motaweh MM, Kleibo IS. Aspirated foreign bodies in the tracheobronchial tree: report of 250 cases. Thorax 1976;31:635-40.

18. McGuirt WF, Holmes KD, Feehs R, Browne JD. Tracheobronchial foreign bodies. Laryngoscope 1988;98:615-8.

19. Mantel K, Butenandt I. Tracheobronchial foreign body aspiration in childhood: a report on 224 cases. Eur J Pediatr 1986; 145:211-6.

20. Saijo S, Tomioka S, Takasaka T, Kawamoto K. Foreign bodies in the tracheobronchial tree: a review of 110 cases. Arch Otorhinolaryngol 1979;225:1-7.

21. Hamilton AH, Carswell F, Wisheart JD. The Bristol Children's Hospital experience of tracheobronchial foreign bodies. Bristol Med Chir J 1989;104:72-4.

22. Inglis AF Jr, Wagner DV. Lower complication rates associated with bronchial foreign bodies over the last 20 years. Ann Otol Rhinol Laryngol 1992;101:61-6.

23. Marsh BR. Teaching bronchoesophagology in otolaryngology–head and neck surgery residencies in the United States. Trans Am Broncho Esophagol Assoc 1989:16-21.

24. Jackson C. Bronchoscopy and esophagoscopy: a manual of peroral endoscopy and laryngeal surgery. Philadelphia, Pa: WB Saunders, 1922:117-25.

25. Jackson C. The life of Chevalier Jackson, an autobiography. New York, NY: Macmillan, 1938.

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MIDDLE EAR MECHANICS IN RESEARCH AND OTOSURGERY

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ADENOVIRUS-MEDIATED GENE THERAPY FOR HEAD AND NECK SQUAMOUS CELL CARCINOMAS

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Advanced head and neck squamous cell carcinomas (HNSCCs) have a poor prognosis despite aggressive multimodal therapy. The goal of our study was to test the feasibility of gene transduction as a novel therapy for head and neck cancer. Three human HNSCC cell lines were transduced in vitro with a replication-deficient recombinant adenovirus containing the lacZ marker gene (Ad2/CMVβgal). Gene transduction efficiency was dependent on multiplicity of infection, duration of exposure to the virus, and viral concentration. Next, the HEp-2 cell line was transduced with an adenoviral vector (Ad.RSV*tk*) containing the herpes simplex virus thymidine kinase (HSV*tk*) gene, which, when expressed, sensitizes transduced cells to ganciclovir (GCV). Subsequent administration of GCV resulted in complete tumor cell killing. These results suggest that adenovirus-mediated transfer of HSV*tk* and administration of GCV has potential as a new therapy for HNSCC.

KEY WORDS — adenovirus, ganciclovir, gene transduction, herpes simplex virus thymidine kinase, squamous cell carcinoma.

INTRODUCTION

Head and neck squamous cell carcinoma (HNSCC) comprises 3% of all adult cancers, and will cause an estimated 12,500 deaths in the United States in 1995.[1] Current therapy for these tumors utilizes an aggressive multimodal approach involving surgery, radiotherapy, and chemotherapy, depending on tumor stage and patient characteristics. The prognosis for stage I or II disease is often good, with a 75% to 90% 5-year survival rate with appropriate therapy. However, even with the most advanced therapy, stage IV disease has only a 15% 5-year survival.[2] Conventional palliative treatments are often toxic and sometimes ineffective. In addition, no effective salvage therapy exists for patients who fail standard treatment.[3] Therefore, new treatments are needed both to improve survival in the long term and to obtain worthwhile, less toxic palliation in the short term.

Gene transfer offers the possibility of new approaches to cancer treatment. Head and neck cancer is particularly well suited for gene transduction strategies. First, its location in the upper aerodigestive tract allows easy access for both vector delivery and assessment of response. Second, a degree of tissue specificity for gene transduction can be achieved by direct injection of the vector into the tumor mass. Finally, metastases mostly occur late in HNSCC tumor progression, making local and regional disease responsible for most of the morbidity and mortality.[4,5] Therefore, any improvement in local and regional control could potentially result in dramatic benefits for patients.

One gene transduction strategy for the treatment of cancer involves the transfer of a "suicide gene" that converts an inactive prodrug into a toxic drug.[6] Subsequent administration of the prodrug results in death of the transduced cell. An example of this strategy is the transfer of the herpes simplex virus thymidine kinase (HSV*tk*) gene followed by administration of ganciclovir [9-(1,3-dihydroxy-2-propoxymethyl) guanine], a purine analog. When the HSV*tk* gene is expressed, its product phosphorylates ganciclovir (GCV). Normal mammalian thymidine kinases are unable to phosphorylate GCV, but are able to phosphorylate monophosphate GCV into triphosphate GCV. Triphosphate GCV causes cell death, presumably by interfering with DNA synthesis.[7]

Recent studies using retroviral vectors to deliver the HSV*tk* gene have shown the HSV*tk*-GCV system to be efficacious against animal models of brain and pancreatic cancer.[8,9] However, the adenovirus may be a more effective vector to deliver genes for the treatment of HNSCC. Unlike retrovirus, it is relatively easily grown and purified to high titer, and infects both dividing and nondividing cells.[10] Infection and gene expression are efficient in most cell

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types, resulting in a high percentage of transduced cells,[5,11,12] and lower doses of GCV may be required.[13] Early studies of adenovirus-mediated gene transfer into humans and nonhuman primates have shown it to be minimally toxic.[14,15]

Therefore, we hypothesized that adenovirus-mediated transfer of HSV*tk* and administration of GCV may be an effective new therapy for head and neck cancer. To test this hypothesis, we first evaluated the ability of the adenovirus vector to transfer foreign genes into head and neck carcinoma cells. Our results demonstrate that the adenovirus vector can efficiently transfer foreign genes into human HNSCC cell lines in vitro. We then found that adenovirus-mediated transfer of HSV*tk* followed by administration of GCV results in dramatic tumor cell killing in HNSCC cell lines. These results suggest that adenovirus-mediated HSV*tk* transduction should be explored as a potential therapy of head and neck cancer.

MATERIALS AND METHODS

Adenovirus Vectors. The production of recombinant replication-deficient adenoviral vectors has been described elsewhere.[13,16-18] Ad2/CMVβgal was a gift from Alan E. Smith, PhD (Genzyme, Cambridge, Mass) and contains the cDNA for β-galactosidase preceded by the cytomegalovirus promoter. Ad2/CFTR-2, also a gift from Alan E. Smith, PhD, contains the cDNA for the cystic fibrosis transmembrane regulator regulated by the viral E1A promoter.

Ad.RSV*lacZ* was constructed from an adenovirus type 5 mutant that lacks most of the sequences in the E1A and E1B regions and a portion of the E3 region, and contains the lacZ gene and the Rous sarcoma virus promoter. Ad.RSV*tk* is identical to Ad.RSV*lacZ* with the exception of the HSV*tk* gene replacing the lacZ gene. Each virus was propagated in HEK293 cells and titers were quantified by HEK293 plaque assays or by end point dilution using anti-adenovirus antibody labeled with fluorescein isothiocyanate.

Cell Culture. Three human HNSCC cell lines were obtained from American Type Culture Collection, Rockville, Md: HEp-2 (laryngeal epidermoid cancer), FaDu (pharyngeal squamous cell carcinoma), and SCC-4 (tongue squamous cell carcinoma). All were grown in their respective recommended media.

Adenovirus Infection. Experiments were performed in 24-well plates (Costar, Cambridge, Mass), at a density of 10,000 cells per well. Standard adenoviral exposure conditions consisted of virus suspended in 0.5 mL cell culture medium, at a multiplicity of infection (MOI) of 500, for 3 hours. The viral suspension was then aspirated and replaced with fresh medium. Cells were incubated for 48 hours, then fixed and assayed for lacZ expression. Individual experiments varied the exposure time, the volume of medium used to suspend the virus, or the MOI (0 to 10,000 virions per cell). The other two variables in each experiment were assigned to standard conditions.

Xgal Staining. Cells were fixed with 1.8% formaldehyde and 2% glutaraldehyde for 5 minutes at 4°C. Next, a solution of 1.0 mg/mL Xgal (5-bromo-4-chloro-3-indonyl-β-D-galactopyranoside, Boehringer Mannheim, Indianapolis, Ind) in phosphate-buffered saline (pH 7.8) was added to each well. The cells were incubated for 16 hours at 37°C without carbon dioxide. For each well, 500 cells were counted by light microscopy and the percentage of cells with blue-stained nuclei was determined. The average of three wells was determined for each variable.

FDG Cell Counting Assay. Fluorescein di-β-D-galactopyranoside (FDG, Molecular Probes, Eugene, Ore) was loaded into cells by hypotonic shock for 30 minutes, and stopped with 1.8 mL of ice-cold medium with 1 mmol/L PETG (phenyl-β-D-thiogalactoside). Fluorescence from 10,000 individual cells was analyzed by fluorescence-activated cell analysis (FACScan, Lysis II software, Becton Dickinson, San Jose, Calif). The percentage of cells positive for β-galactosidase activity was determined by subtracting the fluorescence of the control cells that were not treated with Ad2/CMVβgal.

*Cytotoxicity Studies With Ad.RSV*tk *and GCV.* We plated 1 x 10^6 cells of the HEp-2 line into 25 cm^2 cell culture flasks (Costar) and allowed them to adhere over 16 hours. The cells were then exposed to Ad.RSV*tk* for 4 hours in 2 mL of medium at varying MOIs. Negative controls were either not exposed to virus or exposed to an identical MOI of Ad.RSV*lacZ*. Following exposure, the viral-containing medium was removed, flasks were rinsed with fresh culture medium, 0.025% trypsin with ethylenediaminetetraacetic acid was added for 5 minutes, and cells were recovered by centrifugation. Two hundred cells per well were placed into 6 well plates (Costar) and allowed to adhere for 16 hours. Appropriate wells were then incubated with 20 μmol/L GCV for 24 hours. The GCV-containing medium was removed, the cells were washed, and 3 mL per well fresh medium was added. After 7 days, cell colonies were stained with crystal violet and counted.

RESULTS

Ability of Adenovirus to Transfer Foreign Genes to HNSCC Cell Lines. To determine if an adenovirus vector could mediate foreign gene transfer to HNSCC,

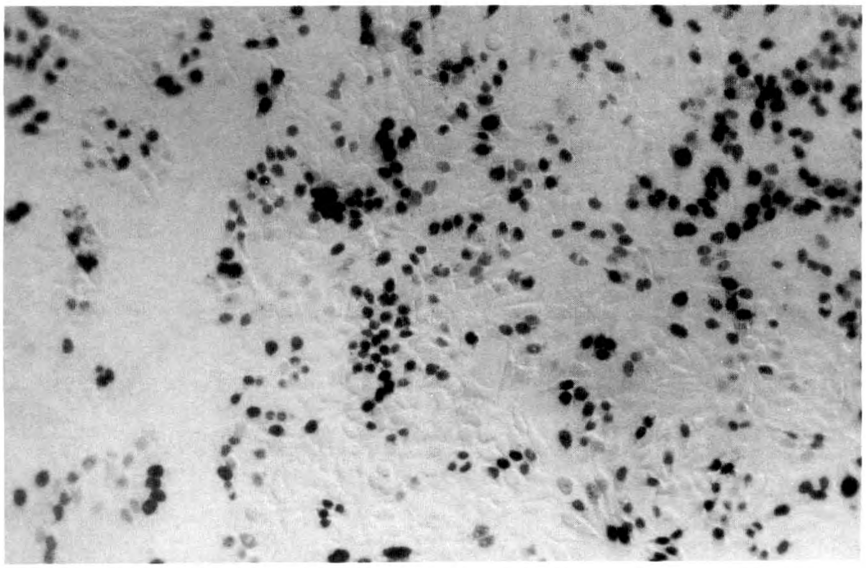

Fig 1. Light microscope assay for expression of lacZ gene. Cell line HEp-2 was exposed to Ad2/CMVβgal (multiplicity of infection [MOI] of 500 infectious units per cell). Expression of lacZ gene was determined by assaying for β-galactosidase activity by means of Xgal. Positive cells demonstrate dark-stained nuclei.

the cell lines HEp-2, FaDu, and SCC-4 were exposed to Ad2/CMVβgal at an MOI of 500 for 3 hours. Expression of the lacZ gene was determined 48 hours after exposure by analysis of Xgal staining for β-galactosidase activity. LacZ gene expression was clearly evident in the cells exposed to Ad2/CMVβgal (Fig 1). Thirty-seven percent ± 4% of HEp-2 ($p < .01$), 35% ± 25% of FaDu ($p < .05$), and 36% ± 7% of SCC-4 ($p < .01$) cells were positive; this finding indicates that the adenovirus could transfer the lacZ marker gene to HNSCC cell lines. Staining was predominantly localized to the nucleus, as expected, because of the fusion of a nuclear localization signal to the lacZ gene. The lacZ gene was not expressed in cells that were not exposed to adenovirus, nor in cells exposed to an adenovirus containing the gene for the cystic fibrosis transmembrane regulator (Ad2/CFTR-2). Therefore, endogenous β-galactosidase activity was not present in these cells under our reaction conditions, and did not occur because of adenovirus alone.

The light microscopic assay using Xgal to determine lacZ gene expression has been shown to underreport transduction efficiency.[16] Therefore, we compared the microscopic assay to a very sensitive fluorescent assay using FDG. When cleaved by β-galactosidase, FDG produces fluorescein, which can be detected by a fluorescence-activated cell sorter (FACS). At an MOI of 500, 34% of HEp-2 cells expressed the lacZ gene by the Xgal assay, and 44% of cells were positive by the FDG-FACS assay (average of two experiments). Again, no expression was detected in unexposed cells or cells exposed to Ad2/CFTR-2. Although the FDG-FACS assay appeared to be more sensitive, the difference was not significant.

Dependence of Gene Transduction on MOI, Virus Exposure Time, and Concentration. We next sought to determine clinical parameters in optimizing gene transduction by investigating MOI, exposure time, and virus concentration. To define optimal MOI, the cell lines were exposed to Ad2/CMVβgal at various MOIs (0 to 10,000) for 3 hours, and LacZ gene expression was determined by Xgal staining for β-galactosidase activity 48 hours later (Fig 2A). No staining occurred in cells not exposed to the adenovirus vector. Significant staining over baseline was seen at an MOI of 50 for SCC-4 and HEp-2 ($p < .01$). There was a clear increase over baseline at the same MOI for FaDu, but it did not reach statistical significance. Staining increased to 35% to 40% of cells at an MOI of 500, and to 84% to 93% at an MOI of 10,000 for each cell line.

To determine the effect of exposure time on gene transfer, the cell lines FaDu and HEp-2 were exposed to Ad2/CMVβgal at an MOI of 500 for various exposure times (1.5 to 48 hours), and lacZ gene expression was determined 48 hours later. As exposure time increased from 1.5 to 24 hours, staining increased from 40% to 95% for FaDu ($p = .03$), and from 31% to 87% for HEp-2 ($p < .01$, Fig 2B). Most of the increase in staining occurred during the first 6 hours, and then increased gradually from 6 to 24 hours. Staining did not increase significantly after 24 hours.

We also evaluated the effect of virus concentration. The cell lines FaDu and HEp-2 were exposed to Ad2/CMVβgal for 3 hours at an MOI of 500, suspended in various amounts of cell culture medium (0.1 to 1.0 mL). As the volume of suspension increased from 0.1 to 1.0 mL, staining decreased linearly from 71% to 29% for FaDu ($p < .05$) and from 79% to 32% for HEp-2 ($p < .05$, Fig 2C).

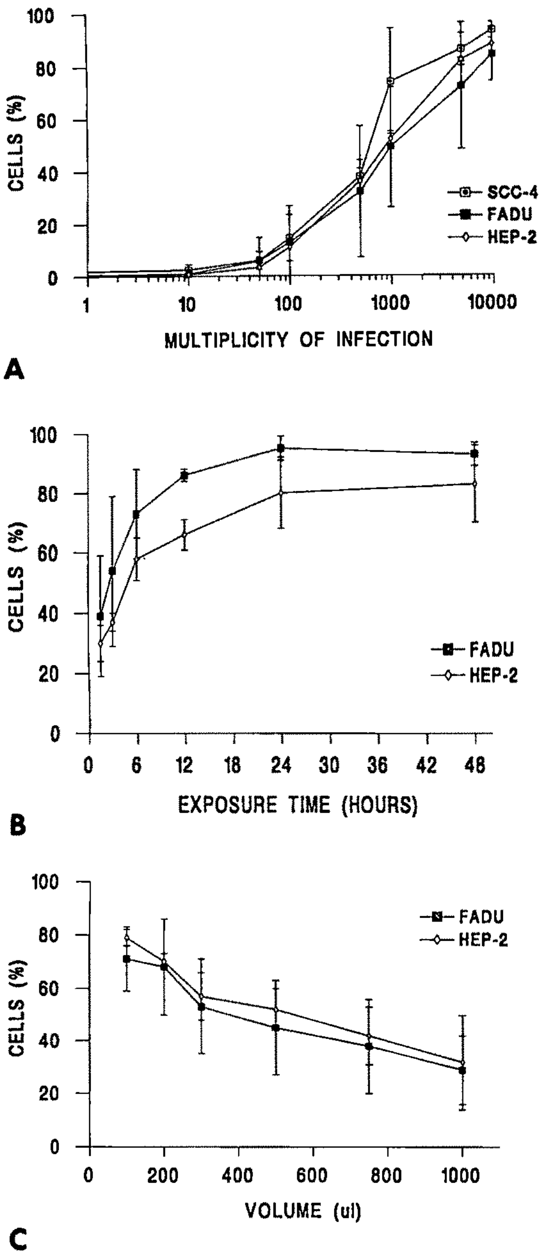

Fig 2. Variables in lacZ gene transduction. A) Effect of MOI on lacZ transduction. Cell lines SCC-4, FaDu, and HEp-2 were exposed to Ad2/CMVβgal for 3 hours at various MOIs. β-Galactosidase activity was determined 48 hours later. B) Effect of adenovirus exposure time on expression of lacZ gene. Cell lines FaDu and HEp-2 were exposed to Ad2/CMVβgal at MOI of 500 for various durations (0, 1.5, 3, 6, 12, 24, 48 hours), and β-galactosidase activity was determined 48 hours later. C) Effect of virus concentration on expression of lacZ gene. FaDu and HEp-2 were exposed to Ad2/CMVβgal for 3 hours at MOI of 500 suspended in various amounts of cell culture medium (100 to 1,000 μL per well). β-Galactosidase activity was determined 48 hours later. Each graph represents average of three experiments performed in triplicate.

*Cell Killing With HSV*tk *and GCV.* Having defined the optimal conditions for gene transfer, we next sought to determine if transfer of the HSV*tk* gene followed by administration of GCV would result in tumor cell killing. Since our adenovirus vector containing the HSV*tk* gene is driven by the RSV promoter (Ad.RSV*tk*), we first examined the ability of the RSV promoter to drive foreign gene expression. HEp-2, FaDu, and SCC-4 cells were exposed to Ad.RSV*lacZ* for 3 hours in 0.5 mL at various MOIs; expression was determined with Xgal as before. The proportion of cells positive for LacZ gene expression was similar to that obtained with Ad2/CMVβgal for the HEp-2 and SCC-4 cell lines. The proportion of positive cells was lower in the FaDu cell line. Significant staining did not occur until an MOI of 100, increasing to only 4% ± 1% of cells at an MOI of 500, and to 49% ± 2% at 10,000.

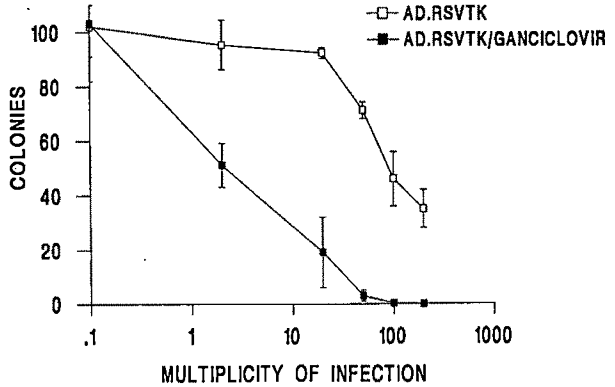

Fig 3. Tumor cell killing with Ad.RSV*tk* and GCV. HEp-2 cells were exposed to Ad.RSV*tk* for 4 hours at various MOIs (0, 2, 20, 50, 100, 200 infection units per cell) and seeded into six-well plates (200 cells per well). Sixteen hours later, cells were either exposed to 20 μmol/L ganciclovir (GCV) for 24 hours (open squares), or not exposed to GCV (closed squares). Seven days later, cell colonies were stained with crystal violet and counted. Each line represents average of three experiments performed in triplicate.

For cell killing experiments, the HEp-2 cell line was exposed to Ad.RSV*tk* for 4 hours at various MOIs (2, 20, 50, 100, 200) in high concentration. Immediately following transduction, the cells were harvested, replated at low density (200 cells per 35 mm^2 well), and allowed to adhere for 16 hours. One group of cells was then exposed to 20 μmol/L GCV for 24 hours; another group was not. Both groups were allowed to grow in culture medium without GCV for 7 additional days. Cytotoxicity was determined by comparing the number of colonies formed under experimental conditions versus those in negative controls.

As shown in Fig 3, tumor cell killing was achieved with adenoviral transduction of HSV*tk* followed by GCV exposure. Fifty percent ± 8% tumor cell killing occurred at an MOI of 2, which increased to 82% ± 13% at an MOI of 20 and 100% at an MOI of 100. For

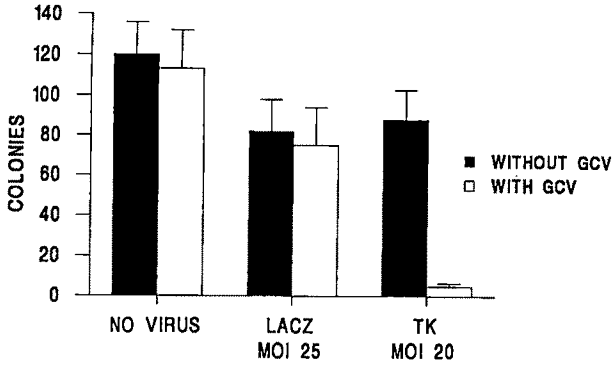

Fig 4. Tumor cell killing compared to negative controls. HEp-2 cells with no viral exposure (column 1), Ad.RSV*lacZ* exposure (MOI 25, column 2), or Ad.RSV*tk* exposure (MOI 20, column 3), with (white) and without (black) subsequent 20 μmol/L GCV for 24 hours. Cell colonies were counted 7 days later. Each column represents average of three experiments performed in triplicate.

cells that were exposed to Ad.RSV*tk* but not to GCV, significant tumor cell killing did not occur below an MOI of 100 ($p < .05$). There was a 50-fold difference in the MOI needed to achieve 50% cell killing between cells exposed to GCV versus those not exposed to GCV. Similar results were obtained with the FaDu cell line (data not shown).

The Xgal assay for β-galactosidase activity was used to determine Ad.RSV*lacZ* transduction efficiency under these reaction conditions. At an MOI of 25, 58% of Ad.RSV*lacZ*-exposed cells stained blue with Xgal. The increase in gene transduction efficiency over previous experiments (Fig 3) can be attributed to the increase in viral concentration and exposure time used in this set of experiments. At an MOI of 20, 96% of cells exposed to Ad.RSV*tk* and GCV were killed; this finding indicates gene transduction to 96% of cells. The apparent difference in gene transduction efficiency between Ad.RSV*lacZ* and Ad.RSV*tk* is probably due to the greater sensitivity of the colony-forming assay versus the Xgal assay. A bystander effect is unlikely under these experimental conditions (see Discussion).

To further investigate toxicity associated with adenoviral infection and transduction, cell killing was determined for HEp-2 cells under the following six conditions: no viral exposure, exposure to Ad.RSV*lacZ* (MOI 25), or exposure to Ad.RSV*tk* (MOI 20), with and without the subsequent administration of 20 μmol/L GCV. As shown in Fig 4, there was no significant toxicity associated with administration of GCV alone (column 1). Adenoviral transduction of the lacZ marker gene resulted in a 31% reduction in cell colonies (column 2, Ad.RSV*lacZ* MOI 25). Administration of GCV did not affect Ad.RSV*lacZ*-transduced cells. The toxicity associated with Ad.RSV*tk* transduction alone at an MOI of 20 (27%) was similar to that with Ad.RSV*lacZ* transduction alone. At higher MOIs, Ad.RSV*tk* was more toxic than Ad.RSV*lacZ* (data not shown).

DISCUSSION

Our results demonstrate that an adenovirus vector can transfer foreign genes into human HNSCC cell lines in vitro. Furthermore, the percentage of cells transduced is dependent on the MOI, the duration of exposure to the vector, and the concentration of the vector. Maximizing these conditions resulted in transduction and expression of the lacZ gene in nearly 100% of the cells. Despite different sites of origin (tongue, pharynx, and larynx), the percentage of cells transduced was similar at each MOI for each cell line. Therefore, the adenovirus vector can be equally useful in transferring foreign genes into HNSCCs of different lineages. Expression of the transferred gene occurred with adenovirus vectors incorporating either a cytomegalovirus or Rous sarcoma virus promoter. Gene transfer can be flexible, then, in the utilization of either promoter.

Transfer of the HSVtk gene and subsequent GCV exposure resulted in dramatic tumor cell killing. Significant tumor cell killing occurred with GCV at Ad.RSV*tk* MOIs as low as 20, and 100% killing occurred at an MOI of 100. Toxicity due to viral exposure alone was seen at an MOI of 50 and greater; Ad.RSV*tk* was more toxic than Ad.RSV*lacZ* at higher MOIs. The cause of the toxicity is not clear, but it may be due to viral protein production or infection by multiple viral particles. It is also unclear why Ad.RSV*tk* is more toxic than Ad.RSV*lacZ* at high MOIs; however, similar results have been documented in a previous study.[13]

We measured cell killing by a colony-forming assay, which has some advantages over standard metabolic assays. Metabolic assays may not differentiate cytotoxic and cytostatic drug effects. By allowing cells to grow for 7 days following a brief exposure to GCV, the difference between the number of colonies formed in experimental and negative control conditions measures true cytotoxicity. In addition, metabolic cooperation between cells that are in contact, which has been suggested to mediate the "bystander effect,"[19,20] is not possible at the low plating density used in this assay. Though we cannot rule out cell killing through the extracellular release of toxic metabolites during cell death or apoptosis, this seems unlikely because of the low number of cells and the relatively large volume of medium. Therefore, tumor cell killing in vivo will likely be as good as or better than that measured by the colony-forming assay in vitro at these MOIs. Finally, tumor cell killing was measured at a clinically achievable GCV concentration (20 μmol/L).[21] No decrease in the efficiency of cell killing was noted in an experiment performed at a 10 μmol/L GCV concentration (data not shown).

These findings suggest that the Ad.RSV*tk*-GCV system may be appropriate for future gene transduction strategies aimed at the treatment of HNSCC. First, adenovirus-mediated gene transfer is highly efficient. Although gene transfer to 100% of cells may not be possible in vivo, previous studies have demonstrated stable cell killing when as few as 10% to 20% of cells were transduced with HSV*tk* in vitro.[11,20] Second, gene expression in vitro is stable during the first 4 days following gene transfer (data not shown). This would allow sufficient time for GCV administration, tumor penetration, and conversion to its active form. Specificity of gene transduction to tumor cells and not normal epithelial cells may be a potential problem. However, a large degree of tumor cell specificity may be achieved by direct injection of the vector into the tumor mass, and by the fact that phosphorylated GCV affects mainly cells undergoing DNA synthesis. Head and neck squamous cell carcinomas are typically rapidly growing tumors surrounded by relatively quiescent fibroblasts, myocytes, and endothelium. Therefore, we would predict little toxicity to normal, nondividing surrounding cells, with most, if not all, toxicity confined to the tumor. As toxicity to surrounding normal tissue has not been tested, however, it is possible that improvements in our targeting strategy will be necessary for the ultimate success of adenovirus-mediated gene therapy for HNSCC.

In conclusion, we have demonstrated that an adenovirus vector can efficiently transfer foreign genes to human HNSCC cell lines, and that transfer of the HSV*tk* gene followed by administration of GCV results in tumor cell killing in vitro. This system holds promise as a novel therapy for HNSCC.

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REFERENCES

1. Wingo PA, Tong T, Bolden S. Cancer statistics, 1995. CA Cancer J Clin 1995;45:8-30.

2. Von Hoff DD. Head and neck cancer. In: Stein JH, ed. Internal medicine. New York, NY: Mosby–Year Book, 1994:943-7.

3. Million RR, Cassisi NJ, Clark JR. Cancer of the head and neck. In: Devita VT Jr, Hellman S, Rosenberg SA, eds. Cancer, principles and practice of oncology. Philadelphia, Pa: JB Lippincott, 1989:488-590.

4. Merino OR, Lindberg RD, Fletcher GH. An analysis of distant metastases from squamous cell carcinoma of the upper respiratory and digestive tracts. Cancer 1977;40:145-51.

5. Clayman GL, El-Naggar AK, Roth JA, et al. *In vivo* molecular therapy with *p53* adenovirus for microscopic residual head and neck squamous carcinoma. Cancer Res 1995;55:1-6.

6. Vile R, Russell SJ. Gene transfer technologies for the gene therapy of cancer. Gene Ther 1994;1:88-98.

7. Cheng YC, Grill SP, Dutschman GE, Nakayama K, Bastow KF. Metabolism of 9-(1,3-dihydroxy-2-propoxymethyl)guanine, a new anti-herpes virus compound, in herpes simplex virus–infected cells. J Biol Chem 1983;258:12460-4.

8. Ram Z, Culver KW, Walbridge S, Blaese RM, Oldfield EH. *In situ* retroviral-mediated gene transfer for the treatment of brain tumors in rats. Cancer Res 1993;53:83-8.

9. DiMaio JM, Clary BM, Via DF, Coveney E, Pappas TN, Lyerly HK. Directed enzyme pro-drug gene therapy for pancreatic cancer in vivo. Surgery 1994;116:205-13.

10. Mulligan RC. The basic science of gene therapy. Science 1993;260:926-32.

11. Smythe WR, Hwang HC, Amin KM, et al. Use of recombinant adenovirus to transfer the herpes simplex virus thymidine kinase (*HSVtk*) gene to thoracic neoplasms: an effective *in vitro* drug sensitization system. Cancer Res 1994;54:2055-9.

12. Chen SH, Shine HD, Goodman JC, Woo SL. Gene therapy for brain tumors: regression of experimental gliomas by adenovirus-mediated gene transfer in vivo. Proc Natl Acad Sci USA 1994;91:3054-7.

13. Shewach DS, Zerbe LK, Hughes TL, Roessler BJ, Breakefield XO, Davidson BL. Enhanced cytotoxicity of antiviral drugs mediated by adenovirus directed transfer of the herpes simplex virus thymidine kinase gene in rat glioma cells. Cancer Gene Ther 1994;1:107-12.

14. Simon RH, Engelhardt JF, Yang Y, et al. Adenovirus-mediated transfer of the CFTR gene to lung of nonhuman primates: toxicity study. Hum Gene Ther 1993;4:771-80.

15. Welsh MJ, Smith AE, Zabner J, et al. Cystic fibrosis gene therapy using an adenovirus vector: in vivo safety and efficacy in nasal epithelium. Hum Gene Ther 1994;5:209-19.

16. Zabner J, Couture LA, Smith AE, Welsh MJ. Correction of cAMP-stimulated fluid secretion in cystic fibrosis airway epithelia: efficiency of adenovirus-mediated gene transfer *in vitro*. Hum Gene Ther 1994;5:585-93.

17. Rich DP, Couture LA, Cardoza LM, et al. Development and analysis of recombinant adenoviruses for gene therapy of cystic fibrosis. Hum Gene Ther 1993;4:461-76.

18. Davidson BL, Allen ED, Kozarsky KF, Wilson JM, Roessler BJ. A model system for *in vivo* gene transfer into the central nervous system using an adenoviral vector. Nat Genet 1993;3:219-23.

19. Wan LB, Parysek LM, Warnick R, Stambrook PJ. *In vitro* evidence that metabolic cooperation is responsible for the bystander effect observed with HSV *tk* retroviral gene therapy. Hum Gene Ther 1993;4:725-31.

20. Freeman SM, Abboud CN, Whartenby KA, et al. The "bystander effect": tumor regression when a fraction of the tumor mass is genetically modified. Cancer Res 1993;53:5274-83.

21. Laskin OL, Stahl-Bayliss CM, Kalman CM, Rosecan LR. Use of ganciclovir to treat serious cytomegalovirus infections in patients with AIDS. J Infect Dis 1987;155:323-7.

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ONLAY BONE AUGMENTATION WITH AN OSTEOINDUCTIVE IMPLANT

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The repair of contour defects of craniofacial bone can be accomplished by several methods, including autogenous bone grafts, demineralized bone, and alloplastic materials. The objective of this study was to assess a biodegradable, xenogeneic, osteoinductive implant for craniofacial onlay bone augmentation. Twelve New Zealand White rabbits each had craniofacial onlays consisting of three experimental materials: 1) autograft; 2) allogeneic, demineralized block implant; and 3) partially purified osteoinductive protein (osteogenin) with allogeneic collagen and 50:50 poly(DL-lactide-co-glycolide). Implants with host bone were recovered after 20 weeks and assessed by histomorphometric methods. There was no evidence of adverse local reaction to the three treatments. No significant difference in bone replacement or bone density resulting from each of the implant types was demonstrated. In conclusion, the osteoinductive implants were well tolerated, and over the 20-week period they appeared to maintain their contour as onlays.

KEY WORDS — bone graft, osteoconduction, osteoinduction.

INTRODUCTION

The repair of contour defects of craniofacial bone can be accomplished by several methods. Autogenous split-calvarial (membranous) bone grafts are rapidly becoming the procedure of choice.[1] Recent experimental evidence has shown increased volume survival of autogenous membranous versus autogenous endochondral onlay grafts.[2-4] The disadvantages of autogenous bone grafting are the additional procedure required for harvest and the limited amount of bone available.

An ideal "off the shelf" biodegradable, osteoinductive implant material would not have the same immunogenic and infectious risks, and there would be no donor site morbidity. Ideally, it would result in predictable and lasting bone replacement.

Allogeneic bank bone is inferior to autogenous bone grafts, because it is slower to incorporate, vascular penetration is slower and less dense, there is less perivascular new bone formation, and there is more surface bone replacement.[5] Furthermore, allogeneic bank bone is potentially immunogenic and there is concern about the transmission of viral pathogens.[6,7]

Another alternative to split calvarial grafts is demineralized allogeneic bone, which has a significant degree of osteoinductive potential.[8-10] In a recent clinical study, however, these implants were shown to have a long-term resorption rate of approximately 50%.[11]

The objective of this study was to assess the potential use of a biodegradable xenogeneic osteoinductive implant as a craniofacial onlay and compare this with allogeneic demineralized membranous bone implants and with autogenous membranous bone onlay grafts. Survival, maintenance of implanted volume and shape, and the extent of bony replacement were assessed.

HISTORICAL BACKGROUND

Urist[12] in 1965 first clearly demonstrated that an implant of demineralized bone will result in the formation of new osseous tissue. Reddi and Huggins[13] in 1972 demonstrated that bone formation in response to demineralized bone was the result of endochondral ossification with fibroblasts (or possibly undifferentiated mesenchymal cells) transformed first into chondroblasts and then into osteoblasts.

In 1981, Sampath and Reddi[14] extracted a fraction of proteins from demineralized bone matrix that demonstrated osteogenic activity only when in the presence of bone matrix. Only those proteins with a

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molecular weight less than 50 kd were biologically active. Proteins larger than 50 kg seemed to have an inhibitory effect. In 1983, Sampath and Reddi[15] demonstrated the homology of osteoinductive proteins from several different animal sources.

In 1984, Urist et al[16] reported the isolation of bovine bone morphogenetic protein, which has a xenogeneic capacity to stimulate osteogenesis. In 1987, Sampath et al[17] isolated and purified a protein they named osteogenin (now referred to as bone morphogenetic protein–3). Osteogenin is a member of the transforming growth factor–β superfamily, which includes bone morphogenetic proteins 1 through 8.[18] For effective therapeutic application, the bone morphogenetic proteins, such as osteogenin, require a delivery system.[19]

Osteoinductive preparations have been used in a variety of laboratory and clinical applications in the craniofacial region. Demineralized bone has been used for both interpositional and onlay grafting in laboratory animals and in humans.[6-9,20] The use of demineralized bone generally results in unacceptable long-term resorption.[9] In 1987, Deatherage and Miller[21] reported the packaging of partially purified osteoinductive proteins in a collagenous implant. This implant elicited rapid endochondral ossification, but was limited in application by its gelatinous consistency when hydrated.

In 1988, Schmitz and Hollinger[22] reported on the use of implants produced by combining allogeneic, demineralized, freeze-dried bone with a biodegradable copolymer of polylactide-polyglycolide. These were implanted into nonhealing calvarial defects in New Zealand White rabbits and demonstrated significant regeneration of trabecular bone.

In 1990, Hollinger et al[23] reported on the use of the same copolymer combined with antigen-extracted autolyzed bone and with bone morphogenetic protein. These implants were placed into nonhealing calvarial defects in nonhuman primates. The antigen-extracted autolyzed bone showed an excellent osseous response, and the copolymer carrier acted as an effective soft tissue spacer before being absorbed and replaced by bone.

MATERIALS AND METHODS

Overview. A total of 12 New Zealand White rabbits were used in this study. Each animal received all three implant types and was its own control. After 20 weeks, the animals were painlessly euthanized, and the implanted area of the frontal bones was assessed for osseous replacement. Histomorphometric methods were used to quantify the degree of bony replacement, and statistical methods were used to analyze the results.

Manufacture of Implants. The autologous membranous bone grafts were harvested by making an incision over the zygomatic arch of each experimental animal and removing a segment of the zygomatic arch measuring approximately 10 × 5 × 3 mm. This was then stripped of periosteum.

The xenogeneic osteoinductive implants were produced by extracting a partially purified bone inductive protein (osteogenin), reconstituting this with inactive collagenous bone matrix,[17] and combining this with a biodegradable carrier: 50:50 poly(DL-lactide-co-glycolide).

The partially purified preparation of osteogenin was prepared in the following manner. Bovine bone powder was demineralized with 0.5N hydrochloric acid (HCl) and washed with water, and the resulting demineralized bone matrix was extracted for 18 hours at 4°C with 4 mol/L guanidine hydrochloride in 50 mL tris-HCl, pH 7.4 (1 g demineralized bone matrix in 30 mL), containing protease inhibitors (5 mmol/L benzamidine hydrochloride, 5 mmol/L *N*-ethylmaleimide, 100 mmol/L 6-aminocaproic acid, and 0.5 mmol/L phenylmethylsulfonyl fluoride). The osteogenin was partially purified by heparin sepharose affinity chromatography and hydroxyapatite chromatography.

The inactive collagenous bone matrix was prepared in the following manner. Rabbit demineralized bone matrix was extracted for 18 hours at 4°C with 4 mol/L guanidine hydrochloride in 50 mmol/L tris-HCl, pH 7.4 (1 g demineralized bone matrix in 30 mL), containing protease inhibitors (5 mmol/L benzamidine hydrochloride, 5 mmol/L *N*-ethylmaleimide, 100 mmol/L 6-aminocaproic acid, and 0.5 mmol/L phenylmethylsulfonyl fluoride). The insoluble rabbit organic matrix remaining after the 4 mol/L guanidine hydrochloride extraction was washed five times with an excess of double-distilled water, dehydrated with ethanol and ether, air dried, and used to reconstitute the osteogenin.[17]

The osteogenin and the inactive collagenous bone matrix was reconstituted in the following manner. One hundred micrograms of osteogenin was suspended in 200 μL phosphate-buffered saline (pH 4) and added to 25 mg of inactive collagenous bone matrix with 0.5 mg chondroitin sulfate. This was then mixed with 1.0 mL of absolute ethanol (prechilled to −70°C) and left at −70°C for 30 minutes, followed by centrifugation at 10,000 rpm in a Beckman Microfuge 12 at 4°C for 15 minutes.

The reconstituted osteogenin was combined with the biodegradable carrier in the following manner.

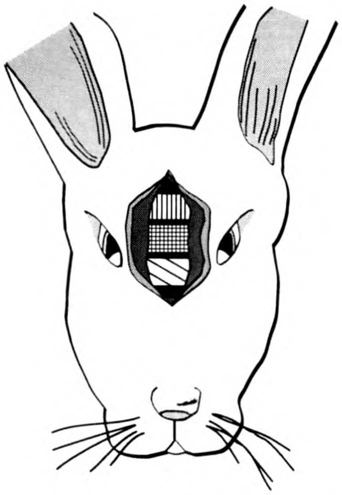

Fig 1. Implants were placed adjacent to each other in caudal to cephalic pattern along rabbit's snout in subperiosteal pocket.

Acetone was used to dissolve the 50:50 poly(DL-lactide-co-glycolide). The reconstituted osteogenin was then added, and the acetone was allowed to evaporate for 48 hours. The implants were then aerated and degassed under vacuum (25°C; 0.20 mm Hg) for 48 hours to remove residual gasses and reactive products.

The demineralized bone implants were prepared by taking fresh membranous bone harvested from the zygomatic arches of donor rabbits used for other purposes. These were defatted in 100% ethanol for 4 hours, decalcified for 24 hours in 0.6N HCl at 4°C, rinsed in 0.1 mol/L phosphate-buffered saline and distilled water, and lyophilized.[17]

Animal Model. The New Zealand White rabbit, a strain of the European rabbit, *Oryctolagus cuniculus*, was selected for this study. This species is anatomically ideal for the study because of its relatively broad, flat snout and forehead that is an ideal location for onlay grafting. A total of 12 rabbits were used in the experiment.

Animal Procedures. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals and the NIH *Guide for the Care and Use of Laboratory Animals*, and the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of Madigan Army Medical Center.

The animals were preanesthetized with glycopyrrolate at a dose of 0.01 mg/kg administered intramuscularly. They were then anesthetized with intramuscular tiletamine 15 mg/kg and zolazepam 15 mg/kg, intubated, and maintained on isoflurane 1% to 2% in oxygen via an endotracheal tube connected to an anesthesia circuit. All of the animals were kept on a recirculating warm water pad to maintain body temperature while anesthetized, and were placed on an identical pad in an intensive care unit cage for 24 hours postoperatively for frequent observation by the animal care staff. In addition, Combipen, at 30,000 IU/kg, was administered intramuscularly as a preoperative antibiotic. All of the rabbits were adult males weighing at least 3.5 kg.

Surgical Procedure. The autogenous zygomatic bone graft was harvested as previously described. The bone graft, the demineralized bone implant, and the osteoinductive implant were carefully measured with calipers prior to implantation. The approximate size of each of the implants was 10 × 5 × 3 mm; however, this was measured precisely with the calipers prior to implantation. A longitudinal incision was made along each animal's snout and forehead, and the periosteum was elevated. The implants were then placed adjacent to each other in a caudal to cephalic pattern along the rabbit's snout in the subperiosteal pocket (Fig 1). The relative positions of the graft and implants were rotated so that they occupied each of the three possible positions in 4 rabbits per position. The wounds were then closed in two layers, with a 4-0 polyglycolic acid suture used to close the periosteum, and a running 4-0 polyglycolic acid suture used to close the skin and subcutaneous tissue.

At the end of 20 weeks, the animals were painlessly euthanized with sodium pentobarbital. Then the frontal bone with the attached ossified implants was harvested with a minimal amount of soft tissue.

The specimens were examined grossly. Calipers were used to carefully measure the length and width of the ossified implants.

The specimens were then placed in 70% alcohol, followed by ascending alcohols. Then the specimens were placed into polymethylmethacrylate for undecalcified sectioning. Serial sections were stained with a modification of Goldner trichrome and von Kossa stains. The specimens were sectioned sagittally with a thickness of 4.5 μm.

Histomorphometric methods were used to measure the bone replacement. A Leica image analysis system with a Zeiss Axiophot microscope was used to measure the cross-sectional area of new bone (trabecular bony area) formed at each implant or graft site. The trabecular bony area was quantitated with a digitizing board and light cursor used to compute the percentage of each microscopic field occupied by new bone. Also, the thickness and width of the ossified implants were carefully measured microscopically.

Data Analysis. The percentage of bone replace-

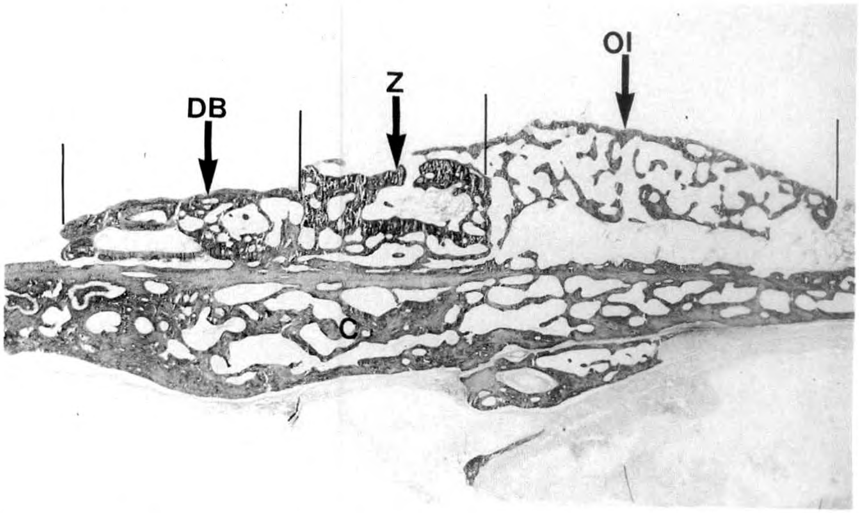

Fig 2. Photomicrograph demonstrates bone resulting from demineralized bone (DB), autogenous zygoma bone graft (Z), and osteoinductive implant (OI) resting on rabbit calvarium (C) (Masson-Goldner trichrome, original ×1.25).

ment and the bone density were calculated from the implant dimensions and the cross-sectional area of the trabecular bone. Bone replacement is defined as the cross-sectional area of the trabecular bone divided by the original cross-sectional area of the implant and expressed as a percent. Bone density is defined as the cross-sectional area of the trabecular bone divided by the final cross-sectional area of the bone resulting from each implant, also expressed as a percent. The change in each dimension (height, width, and thickness) and the change in volume were calculated by comparing the original dimensions of each implant with the dimensions of the resulting bone. Repeated measures analysis of variance was used to test the null hypothesis that there was no difference between the implant types in regard to change in dimension. When a difference was deected, a post hoc Duncan multiple range test was employed to determine significant differences beween the mean measurements of each implant type.

RESULTS

Nine animals survived the full 20-week course of he study. Of the 3 animals that died, 1 died in the mmediate postoperative period, presumably because f the stress of surgery. The other 2 died approximately 6 weeks after placement of the implants of unknown causes apparently not related to the implants.

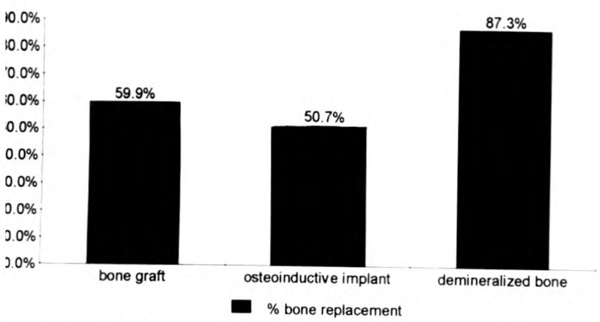

Fig 3. Calculated percent bone replacement of original volume of implants.

At necropsy of the 9 animals surviving the full 20 weeks, it was obvious that hard bony tissue had replaced the osteoinductive implants and the demineralized bone implants. In addition, all of the autogenous bone grafts were adhered to the underlying calvarium. Grossly, the resulting bone approximated the original dimensions of the implants and grafts. There was noted, however, some rounding of the edges and corners.

Histologic examination confirmed the observations made at necropsy. As can be seen from the photomicrograph (Fig 2), there was bony replacement in all three implant types. The calculated percent bone replacements were 59.9% ± 34.6% (SD) for the autogenous membranous bone grafts, 50.7% ± 24.6% for the osteoinductive implants, and 87.3% ± 51.5% for the demineralized bone (Fig 3). Repeated measures analysis of variance was used to test the null hypothesis that the bone densities were not different. No significant difference could be shown, with a p value of .1834.

The calculated percent bone densities were 61.6% ± 28.6% for the autogenous membranous bone grafts, 58.9% ± 48.4% for the osteoinductive implants, and 62.7% ± 29.4% for the demineralized bone. Repeated measures analysis of variance was used to test the null hypothesis that the bone densities were not different. No significant difference could be shown, with a p value of .9543.

The mean percent changes in the length, width and thickness of the demineralized bone implants were −1.2% ± 18.2%, −2.3% ± 13.3%, and 55.5% ± 55.4%, respectively; in the osteoinductive implant they were 1.5% ± 17.5%, 23.3% ± 25.2%, and −17.9% ± 29.2%;

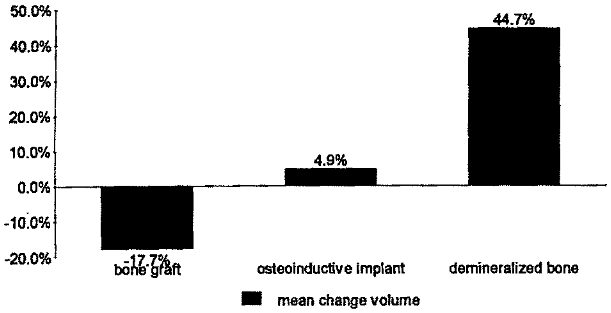

Fig 4. Mean percent change in volume of bone resulting from each implant type relative to implanted volume.

and in the autogenous bone graft they were −10.7% ± 18.7%, −8.3% ± 4.3%, and 1.4% ± 24.7%. Repeated measures analysis of variance revealed a statistically significant difference among the three implant types in all of the dimensions, with p values of .0130, .0258, and .0136 for the length, width, and thickness, respectively. The Duncan multiple range test was used to determine which implant type was different. In regard to length, the osteoinductive implant was significantly different from both the bone graft and the demineralized bone ($p < .05$), which were not significantly different from each other. In regard to width, the bone graft was significantly different from both the osteoinductive implant and the demineralized bone ($p < .05$), which were not significantly different from each other. In regard to thickness, the demineralized bone was significantly different from both the bone graft and the osteoinductive implant ($p < .05$), which were not significantly different from each other.

The mean percent volume changes were for demineralized bone 44.7% ± 50.7%, for the osteoinductive implants 4.9% ± 47.6%, and for the autogenous bone graft −17.7% ± 27.6% (Fig 4). Repeated measures analysis of variance revealed a statistically significant difference among the three implant types, with a p value of .0255. With the Duncan multiple range test, the volume of the demineralized bone was found to be significantly different from those of both the bone graft and the osteoinductive implant ($p < .05$), which were not significantly different from each other.

DISCUSSION

In this study, the xenogeneic osteoinductive implants were produced by taking a partially purified preparation of bovine bone morphogenetic protein, reconstituting it with inactive collagenous bone matrix, and combining this with a biodegradable osteoinductive carrier consisting of 50:50 poly(DL-lactide-co-glycolide). The implants were placed in a subperiosteal location on the foreheads of 12 New Zealand White rabbits. In addition, autogenous membranous bone grafts from the animal's zygomatic arches, and allogeneic demineralized rabbit zygomas were placed alongside the osteoinductive implants as controls (Fig 1). These three implant types were left in their subperiosteal location for a period of 20 weeks, at which time they were harvested and processed by standard histologic methods. Histomorphometric methods were used to quantitate the trabecular bony volume present at the end of the study. In addition, the dimensions of the implanted materials were carefully measured at the beginning and the end of the study to assess the conservation of dimensions and volume.

The short-term results with this implant were quite encouraging. The bone produced as a result of this implant had a bone replacement and density that was not significantly different from that resulting from membranous bone grafts and demineralized bone. In a larger study, it may be possible to show a statistically significant difference in bone replacement and bone density; however, this would be of little clinical relevance if the resulting bone was strong and retained the size and shape over the long term. These results suggest that in the future, similar implants would be a suitable substitute for bone grafts that would avoid donor site morbidity. Furthermore, the dimensions of the resulting bone compared favorably with the original dimensions of the implant. This would be an advantage in aesthetic applications, in which a precise replication of the deficient tissue is important. Purified preparations of bone morphogenetic protein produced from recombinant DNA technology are now available in commercial quantities. This may allow better results with more easily obtainable materials.

There is a liability of assessing three different materials in a contiguous recipient bed. An implant material could conceivably have an effect on the osteoinductive effect of an adjacent implant material. This would have the greatest effect on the width dimension of the bone resulting from the implant located between the other two. This effect was compensated for by varying the relative locations of the implants. A similar model was used by Hardesty and Marsh[2] to evaluate craniofacial onlay bone grafts.

Rabbits are known to have a higher osteogenic potential than animals higher on the phylogenetic tree.[24] For this reason, similar results might not be obtained in primates.

A final consideration is whether the bone produced by osteogenic implants will be durable and not subject to resorption. It has been established that endochondral onlay bone grafts tend to resorb to a significant extent over time, while membranous bone onlay bone grafts tend to be more durable.[2-4] The long-term durability of bone produced from osteogenic implants remains to be seen.

It is clear that induced osteogenesis works. The challenge now is to find the ideal delivery system to hold the osteoinductive proteins in place while bony replacement occurs. This delivery vehicle also must form an osteoconductive scaffold that will be replaced by bone or into which bone can grow. Biodegradable and nonbiodegradable materials, including ceramics,[25-27] biodegradable polymers,[27] and clotted blood,[28] are osteoconductive materials that have been shown to have potential in this area.

Last, osteogenic implants may be useful in conjunction with pedicle flaps for osseous reconstruction. It remains to be established how vigorous and durable an osteogenic response can be elicited in such a totally heterotopic site. Combining osteoinductive implants with pedicle flaps will potentially open new possibilities in the reconstruction of osseous defects in tissues that have been irradiated or otherwise rendered hostile to conventional bone grafting techniques.

CONCLUSIONS

1. The osteoinductive implants were well replaced with bone over a 20-week period, with the bone comparing favorably in regard to density with autogenous membranous bone grafts and demineralized bone.

2. The shape and size of the resulting bone are a reasonably good replication of those of the original osteoinductive implant.

REFERENCES

1. McCarthy JG, Zide BM. The spectrum of calvarial bone grafting: introduction of the vascularized calvarial bone flap. Plast Reconstr Surg 1984;74:10-8.

2. Hardesty RA, Marsh JL. Craniofacial onlay bone grafting: a prospective evaluation of graft morphology, orientation, and embryonic origin. Plast Reconstr Surg 1990;85:5-15.

3. Smith JD, Abramson M. Membranous vs endochondral bone autografts. Arch Otolaryngol 1974;99:203-5.

4. Zins JE, Whitaker LA. Membranous vs. endochondral bone: implications for craniofacial reconstruction. Plast Reconstr Surg 1983;72:778-85.

5. Brown KLB, Cruess RL. Bone and cartilage transplantation in orthopaedic surgery. J Bone Joint Surg [Am] 1982;64:270-9.

6. Horowitz MC, Friedlaender GE. Immunologic aspects of bone transplantation. Orthop Clin North Am 1987;18:227-33.

7. Tomford WW, Mankin JH, Friedlaender GE, Doppelt SH, Gebhardt MC. Methods of banking bone and cartilage for allograft transplantation. Orthop Clin North Am 1987;18:241-7.

8. Kaban LB, Glowacki J. Induced osteogenesis in the repair of experimental mandibular defects in rats. J Dent Res 1981;60: 1356-64.

9. Mulliken JB, Glowacki J. Induced osteogenesis for repair and construction in the craniofacial region. Plast Reconstr Surg 1990;65:553-9.

10. Mulliken JB, Glowacki J, Kaban LB, Folkman J, Murray JE. Use of demineralized allogeneic bone implants for the correction of maxillocraniofacial deformities. Ann Surg 1981;194:366-72.

11. Toriumi DM, Larrabee JF, Walike JW, Millay DJ, Eisele DW. Demineralized bone. Arch Otolaryngol Head Neck Surg 1990;116:676-80.

12. Urist MR. Bone: formation by autoinduction. Science 1965;150:893-9.

13. Reddi AH, Huggins C. Biochemical sequences in the transforming of normal fibroblasts in adolescent rats. Proc Natl Acad Sci USA 1972;69:1601-5.

14. Sampath TK, Reddi AH. Dissociative extraction and reconstitution of extracellular matrix components involved in local bone differentiation. Proc Natl Acad Sci USA 1981;78:7599-603.

15. Sampath TK, Reddi AH. Homology of bone-inductive proteins from human, monkey, bovine, and rat extracellular matrix. Proc Natl Acad Sci USA 1983;80:6591-4.

16. Urist MR, Huo YK, Brownell AG, et al. Purification of bovine bone morphogenetic protein by hydroxyapatite chromatography. Proc Natl Acad Sci USA 1984;81:371-5.

17. Sampath TK, Muthukumaran N, Reddi AH. Isolation of osteogenin, an extracellular matrix–associated bone-inductive protein, by heparin affinity chromatography. Proc Natl Acad Sci USA 1987;84:7109-13.

18. Massague J. The transforming growth factor–beta family. Annu Rev Cell Biol 1990;6:597-641.

19. Kenley RA, Yim K, Abrams J, et al. Biotechnology and bone graft substitutes. Pharm Res 1993;10:1393-401.

20. Ousterhout DK. Clinical experience in cranial and facial reconstruction with demineralized bone. Ann Plast Surg 1985;15: 367-73.

21. Deatherage JR, Miller EJ. Packaging and delivery of bone induction factors in a collagenous implant. Coll Relat Res 1987; 7:225-31.

22. Schmitz JP, Hollinger JO. A preliminary study of the osteogenic potential of a biodegradable alloplastic-osteoinductive alloimplant. Clin Orthop Relat Res 1988;237:245-55.

23. Hollinger JO, Schmitz JP, Mark DE, Seyfer AE. Osseous wound healing with xenogeneic bone implants with a biodegradable carrier. Surgery 1990;107:50-4.

24. Schmitz JP, Hollinger JO. The critical size defect as an experimental model for craniomandibulofacial nonunions. Clin Orthop Relat Res 1986;205:299-308.

25. Nade S, Armstrong L, McCartney E, Baggaley B. Osteogenesis after bone and bone marrow transplantation. Clin Orthop Relat Res 1983;181:255-63.

26. Toriumi DM, East CA, Rosen DM, Chu G, Liu CC, Larrabee WF. Bone-inducing implants in head and neck surgery: an experimental study. Laryngoscope 1991;101:395-404.

27. Hollinger JO, Battistone GC. Biodegradable bone repair materials. Clin Orthop Relat Res 1986;207:290-305.

28. Toriumi DM, Kotler HS, Luxenberg DP, Holtrop ME, Wang EA. Mandibular reconstruction with a recombinant bone-inducing factor. Arch Otolaryngol Head Neck Surg 1991;117: 1101-12.

Ann Otol Rhinol Laryngol 105:1996

IMAGING CASE STUDY OF THE MONTH

MAGNETIC RESONANCE IMAGING OF AN INHALED PEANUT

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INTRODUCTION

A foreign body aspirated in the tracheobronchial tree is one of the high-risk accidents of childhood. Cohen et al[1] reported that a peanut is the most commonly inhaled foreign body. However, its inhalation is sometimes difficult to diagnose without typical symptoms. Previous radiologic methods, including chest radiography, fluoroscopy, computed tomography (CT), and radioisotope imaging, cannot clearly visualize radiolucent foreign bodies such as peanuts.

Recently, magnetic resonance imaging (MRI) has been widely used in otolaryngology. In this report, we present a case in which MRI detected an aspirated peanut in the main stem bronchus. The images provided definitive information for the treating surgeons and graphically demonstrated the problems of the child to facilitate better understanding by his parents. This report discusses the usefulness of MRI in clearly detecting a foreign body in the airway.

CASE REPORT

A 16-month-old boy was hospitalized with an 8-day history of sudden wheezing, constant coughing, and fever without a clear history of foreign body aspiration. The boy had had recurrent bronchial asthma since 12 months of age. On admission, a reduction in breath sounds of the left lung was noted. Chest radiography demonstrated air trapping of the left lung and mediastinal deviation to the right. On expiration, the mediastinum shifted to the right side. On inspiration, it returned to the midline (Fig 1). Therefore, an inhaled foreign body in the left bronchus was suspected and the family was asked to permit bronchoscopy under general anesthesia to confirm and remove the foreign body. However, the family wanted

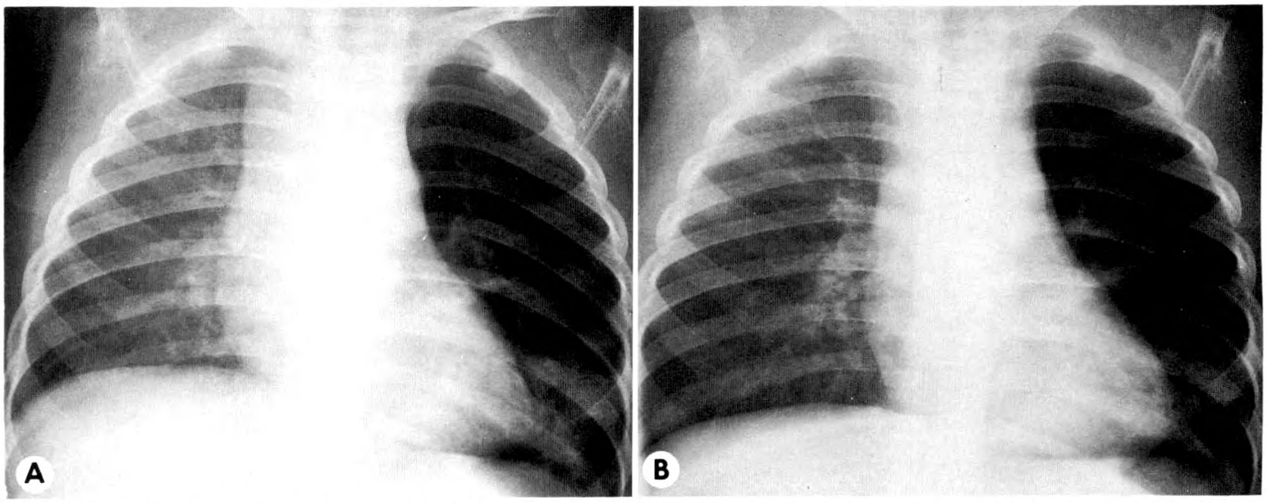

Fig 1. Expiration and aspiration radiography. A) On expiration, air trapping of left lung and mediastinal deviation to right are seen. B) On inspiration, mediastinum swings toward left.

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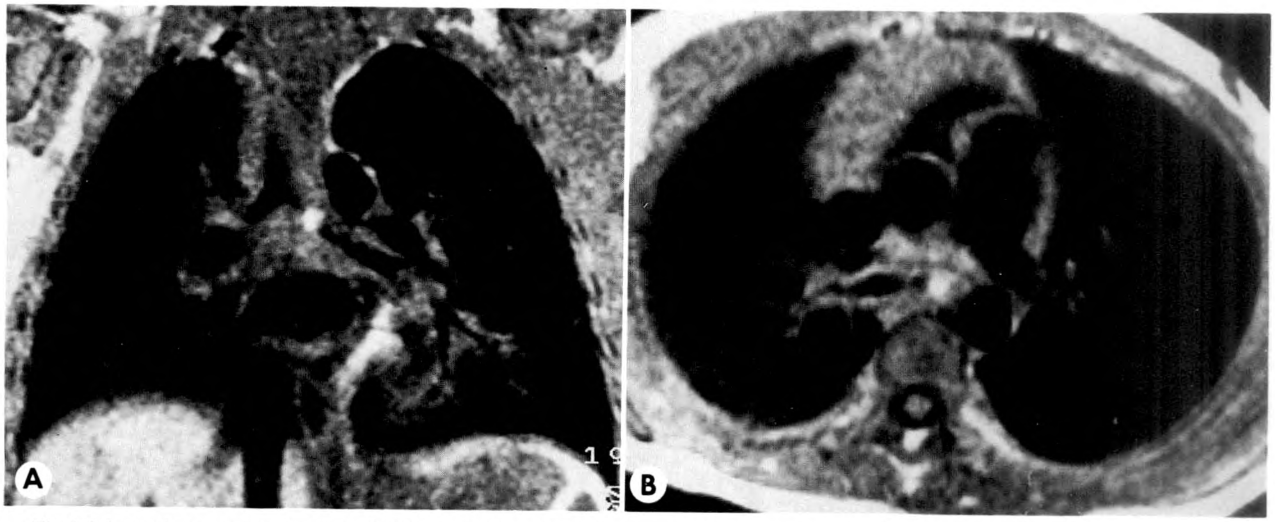

Fig 2. Magnetic resonance images. A) Coronal, T1-weighted, showing high-signal area in left main stem bronchus. B) Axial, T1-weighted.

convincing evidence of the foreign body prior to granting permission. To reconfirm the diagnosis, chest MRI was obtained (Fig 2). The T1-weighted MRI demonstrated not only air trapping of the left lung, but also an intraluminal object of 5-mm diameter with high signal intensity in the left main stem bronchus. The suspected foreign body was confirmed. Informed consent was obtained, and removal was undertaken by means of ventilating bronchoscopy under general anesthesia. Three pieces of a peanut and other material, probably nutshell, were successfully removed (Fig 3). The intraoperative observations correlated well with the MRI findings. The intraoperative and postoperative courses were uneventful. Following this procedure, the patient's wheezing, coughing, and emphysema disappeared.

DISCUSSION

Chest radiography, fluoroscopy, CT, and radionuclide imaging have been used in attempts to identify an inhaled foreign body. The most common type of inhaled foreign body is a piece of nut (55%), followed by food particles (20%) and metallic objects (16.7%).[1] Moskowitz et al[2] reported that only 10% to 15% of foreign bodies are radiopaque. Because a large number of inhaled foreign bodies are radiolucent, radiographic diagnosis mainly depends on secondary changes such as obstructive emphysema, atelectasis, pneumonia, and mediastinal shift.

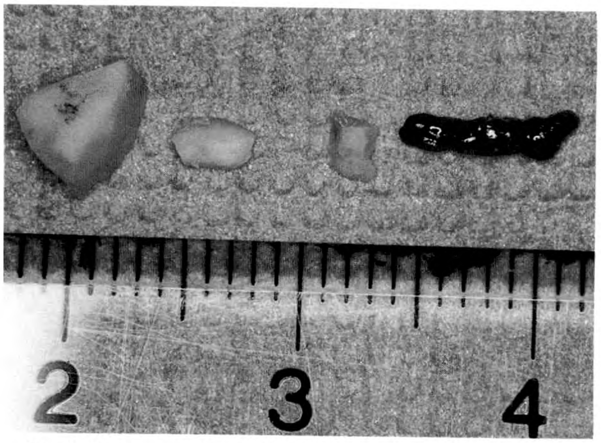

Fig 3. Extracted pieces of peanut and vegetable material (probably nutshell). Scale is in centimeters.

Magnetic resonance imaging is noninvasive and does not involve ionic radiation exposure. It also has major advantages in the high resolution of soft tissue and multiplanar imaging capability.[3,4] As this case report shows, MRI also allows excellent contrast between the air-filled lumen and the peanut. Moreover, coronal MRI superbly delineated the anatomy of the carina and the main stem bronchus.[5]

The disadvantages of MRI in pediatric patients include a relatively long data acquisition time, the need for sedation or anesthesia, the difficulty of monitoring, and the higher cost.[4] In this case, definitive information was obtained with MRI. Direct and graphic demonstration of the foreign body convinced the family of the necessity for bronchoscopy and provided useful preoperative information for safer removal. Magnetic resonance imaging may be a useful adjunct in diagnosis of complicated airway foreign bodies.

CONCLUSION

Magnetic resonance imaging directly visualized an inhaled peanut, which was not visible by conventional radiologic methods. It may be a useful adjunct in diagnosis of complicated foreign body aspiration.

REFERENCES

1. Cohen SR, Herbert WI, Lewis GB Jr, Geller KA. Foreign bodies in the airway. Five-year retrospective study with special reference to management. Ann Otol Rhinol Laryngol 1980; 89:437-42.

2. Moskowitz D, Gardiner LJ, Sasaki CT. Foreign-body aspiration: potential misdiagnosis. Arch Otolaryngol 1982;108: 806-7.

3. Fullerton GD. Physiologic basis of magnetic relaxation. In: Stark DD, Bradley WG Jr, eds. Magnetic resonance imaging. St Louis, Mo: CV Mosby, 1988:36-53.

4. Yanagisawa E. Magnetic resonance imaging. In: Lee KJ, ed. Essential otolaryngology. New York, NY: Medical Examination Publishing Co, 1987:720-32.

5. Hahn D. Trachea. In: Stark DD, Bradley WG Jr, eds. Magnetic resonance imaging. St Louis, Mo: CV Mosby, 1988:823.

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FOURTH INTERNATIONAL SYMPOSIUM ON CHILDHOOD DEAFNESS

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CLINICOPATHOLOGICAL CONSULTATION

LARYNGEAL LYMPHOMA DERIVED FROM MUCOSA-ASSOCIATED LYMPHOID TISSUE

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Extranodal lymphomas account for as many as 40% of non-Hodgkin's lymphomas, and most arise in the gastrointestinal tract, but other mucosal organs may be involved, especially the upper aerodigestive tract. Low-grade B-cell lymphomas arising in the gastrointestinal tract and other mucosae have been found to recapitulate the structure and cytologic features of mucosa-associated lymphoid tissue (MALT). Histologically low-grade MALT lymphomas are characterized by centrocyte-like B-cells with a phenotype similar to that of so-called marginal zone B-cells. Tumors evolving from MALT are generally rare among lymphomas of the upper aerodigestive tract, but a few cases of laryngeal lymphomas derived from MALT have been reported. Primary MALT lymphoma of the larynx should always be considered in tumors with histopathologic features of low-grade B-cell lymphoma, or so-called pseudolymphoma.

INTRODUCTION

Non-Hodgkin's lymphomas (NHLs) arising in tissues or organs other than lymph nodes are designated as extranodal lymphomas. These account for a considerable proportion of all malignant lymphomas. Freeman et al[1] reported up to 40% of all lymphomas to be of extranodal origin. Most extranodal lymphomas arise in the gastrointestinal tract, but other mucosal organs may also be involved, especially the upper aerodigestive tract.[2] It is noteworthy, however, that primary gastric lymphoma, the most frequent subtype of extranodal NHL, occurs in an organ without preexisting mucosa-associated lymphoid tissue (MALT). Histologically, MALT consists of specialized lymphoid tissue protecting permeable mucosal sites that are in direct contact with the external environment. According to Isaacson and Norton,[2] MALT comprises three different compartments: 1) organized lymphoid follicles with a special B-cell zone external to the mantle zone (these B-cells resemble centrocytes in appearance and have therefore been postulated to represent the normal equivalent of the neoplastic lymphocytes found in MALT lymphoma or MALToma[3]); 2) plasma cells and lymphocytes diffusely infiltrating the lamina propria; and 3) intraepithelial lymphocytes, almost all belonging to the T-cell lineage with the phenotype of cytotoxic cells (CD3+, CD8+, CD5+, CD4–).

A diagnostic problem common to all extranodal lymphomas is their distinction from reactive lymphoid infiltrates or localized hyperplasia of MALT. This is often very difficult on the basis of histopathologic findings alone, because malignant lymphoma may simulate the basic infiltration patterns of benign lymphoid infiltrates.[4] Because many malignant lymphomas of mucosal sites follow a relatively benign clinical course, a considerable proportion of MALT lesions were formerly termed pseudolymphoma. It is now possible to discriminate accurately between reactive and neoplastic lymphoid infiltrates in most cases, however, using immunohistochemical and — when necessary — molecular biological techniques (in situ hybridization, polymerase chain reaction, etc). Thus, the term pseudolymphoma can only be applied in very rare instances in which the lesion can be proven to be reactive.

The features of extranodal lymphomas are far less well documented than those of node-based lymphomas. The current classification systems for malignant lymphoma focus on nodal tumors, and the assumption is made that they can be extrapolated to extend to extranodal tumors. However, there are extranodal tumors that have no nodal counterpart, and although a few are mentioned specifically, there is no classification system that covers the whole spectrum of extranodal lymphomas. A summary of the main subtypes in the Updated Kiel Classification of NHL is given in Table 1.[5] Interestingly, a recent classification of NHL proposed by an International Lymphoma Study Group includes extranodal low-

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TABLE 1. EXCERPT FROM UPDATED KIEL CLASSIFICATION SYSTEM OF MALIGNANT NON-HODGKIN'S LYMPHOMAS

- B-cell neoplasms
  - Low-grade NHL
    - Lymphocytic (chronic lymphocytic leukemia)
    - Lymphoplasmacytic, lymphoplasmacytoid (immunocytoma)
    - Plasmacytic (plasmacytoma)
    - Centroblastic, centrocytic
    - Centrocytic (mantle cell lymphoma)
  - High-grade NHL
    - Centroblastic
    - Immunoblastic
    - Lymphoblastic
    - Burkitt's lymphoma
- T-cell neoplasms
  - Low-grade NHL
    - Lymphocytic (chronic lymphocytic leukemia)
    - Small cell, cerebriform (mycosis fungoides)
    - Lymphoepithelioid (Lennert's lymphoma)
    - Angioimmunoblastic
    - T-zone lymphoma
    - Small cell, pleomorphic
  - High-grade NHL
    - Large cell, pleomorphic
    - Immunoblastic
    - Large cell, anaplastic (Ki1+)
    - Lymphoblastic

Information from Lennert and Feller.[5]
NHL — non-Hodgkin's lymphoma.

TABLE 2. LIST OF B-CELL LYMPHOMAS ACCORDING TO REVISED EUROPEAN-AMERICAN LYMPHOMA CLASSIFICATION

- Precursor B-lymphoblastic lymphoma, leukemia
- B-cell chronic lymphocytic leukemia, prolymphocytic leukemia, small lymphocytic lymphoma
- Lymphoplasmacytoid lymphoma
- Mantle cell lymphoma
- Follicular center lymphoma, follicular
  - Grade I
  - Grade II
  - Grade III
- Follicular center lymphoma, diffuse, small cell (provisional)
- Marginal zone B-cell lymphoma
  - Extranodal marginal zone B-cell lymphoma (low-grade B-cell lymphoma of MALT type)
  - Nodal marginal zone B-cell lymphoma (provisional)
  - Splenic marginal zone B-cell lymphoma (provisional)
- Hairy cell leukemia
- Plasmacytoma, myeloma
- Diffuse large B-cell lymphoma
- Primary mediastinal large B-cell lymphoma
- Burkitt's lymphoma
- High-grade B-cell lymphoma, Burkitt-like (provisional)

Information from Harris et al.[6]

grade B-cell lymphomas of MALT type under the category marginal zone B-cell lymphoma (Table 2[6]). The main difference relating to NHL between these two systems lies in the fact that Harris et al[6] make a distinction between precursor neoplasms of B- or T-cell origin (ie, lymphoblastic lymphoma, leukemia) and peripheral B- or T-cell lymphomas, while the Kiel Classification has always differentiated between low-grade and high-grade lymphomas.[7,8]

CONCEPT OF MALT LYMPHOMA

On the basis of observations in cases of immunoproliferative small intestinal disease (IPSID) and primary low-grade B-cell lymphoma of the stomach, Isaacson and Wright[9] suggested that lymphomas arising from MALT constitute a distinct clinicopathological entity. These observations were later extended to include similar malignant lymphomas arising in other mucosal tissues and organs.[10] Histopathologically typical MALToma is defined by the presence of intermediate-size centrocyte-like cells with infiltration of reactive lymphoid follicles, formation of lymphoepithelial lesions, and associated plasmacytic differentiation.[3] The clinical features of MALT lymphomas were found to differ significantly from those of nodal low-grade B-cell lymphomas, in that MALT lymphomas tend to remain localized to their site of origin for a long time, they rarely disseminate to the bone marrow, and they can often be successfully treated by surgical excision.[2] However, the fact that MALT lymphomas only infrequently arise in sites in which MALT is most abundant, and most commonly arise at sites normally devoid of MALT (particularly the stomach) is a serious obstacle to this concept. It is known that the development of MALT lymphomas of salivary glands is always preceded by myoepithelial sialadenitis, and that gastric lymphoma is always associated with chronic *Helicobacter pylori* infection, with acquired MALT always being found in the gastric mucosa. Therefore, acquired MALT appears to be of major importance in the development of MALT lymphomas at sites normally devoid of MALT.[2] It has also recently been shown that a considerable proportion of patients initially considered to have myoepithelial sialadenitis may in fact have a clonal B-cell proliferation, ie, a low-grade NHL.[11]

HISTOPATHOLOGY AND CLASSIFICATION OF MALT LYMPHOMAS

As mentioned above, typical MALT-type low-grade lymphoma consists of medium-size centrocyte-like tumor cells with an irregular nuclear contour. These cells show a characteristic growth pattern, surrounding follicles and infiltrating the epithelium with the formation of lymphoepithelial lesions that are almost pathognomonic. The follicles themselves, although typical of MALT lymphoma, are reactive in nature.[2,10] Plasmacytic differentiation is a common feature of MALT lymphoma, so that it is often difficult to distinguish such tumors from the lymphoplasmacytic variant of immunocytoma, which may also occur as a primary extranodal lymphoma.

Because there are considerable morphologic similarities between the tumor cells of MALT lymphoma and those of two recently defined types of primary nodal lymphoma, namely, monocytoid B-cell lymphoma and marginal zone B-cell lymphoma, it is often impossible to differentiate between these entities on the basis of histopathologic findings alone, and immunohistochemical investigation may be necessary. The tumor cells of MALT lymphomas typically exhibit the following immunophenotype: sIgM+, (cIg+), CD5–, CD19+, CD20+, CD22+.[2] Lack of reactivity for CD5 may be very helpful in distinguishing MALT lymphoma from infiltrates of CD5+ chronic lymphocytic leukemia and node-based mantle cell lymphoma.[6]

There is no doubt that MALT lymphoma may undergo transformation to high-grade malignant lymphoma, with loss of most of its typical histopathologic features, and in a significant proportion of gastrointestinal lymphomas presenting as high-grade tumors, foci of typical low-grade MALT lymphoma can be identified.[12] Without these foci, high-grade MALT lymphomas cannot be reliably classified as being of MALT origin.[2]

LYMPHOMAS OF LARYNX

Lymphoid neoplasms are relatively common in the upper aerodigestive tract. However, in contrast with the situation in the gastrointestinal tract, typical MALT lymphomas are rare in this region[2]; some authors have even denied their existence at this site.[13] Primary lymphoid tumors very rarely occur in the larynx. The most common type of primary laryngeal lymphoma is extramedullary plasmacytoma.[14] Sixty-five cases of NHL with primary involvement of the larynx have been published.[15]

A total of 11 cases designated as pseudolymphoma or lymphoid hamartoma of the larynx were reported up to 1984.[16] This was before the advent of immunohistochemical techniques, so the diagnosis seems to be questionable, at least.[4] It is important to note that no further cases of laryngeal "pseudolymphoma" have been published since the recognition of MALT lymphoma as a distinct entity.

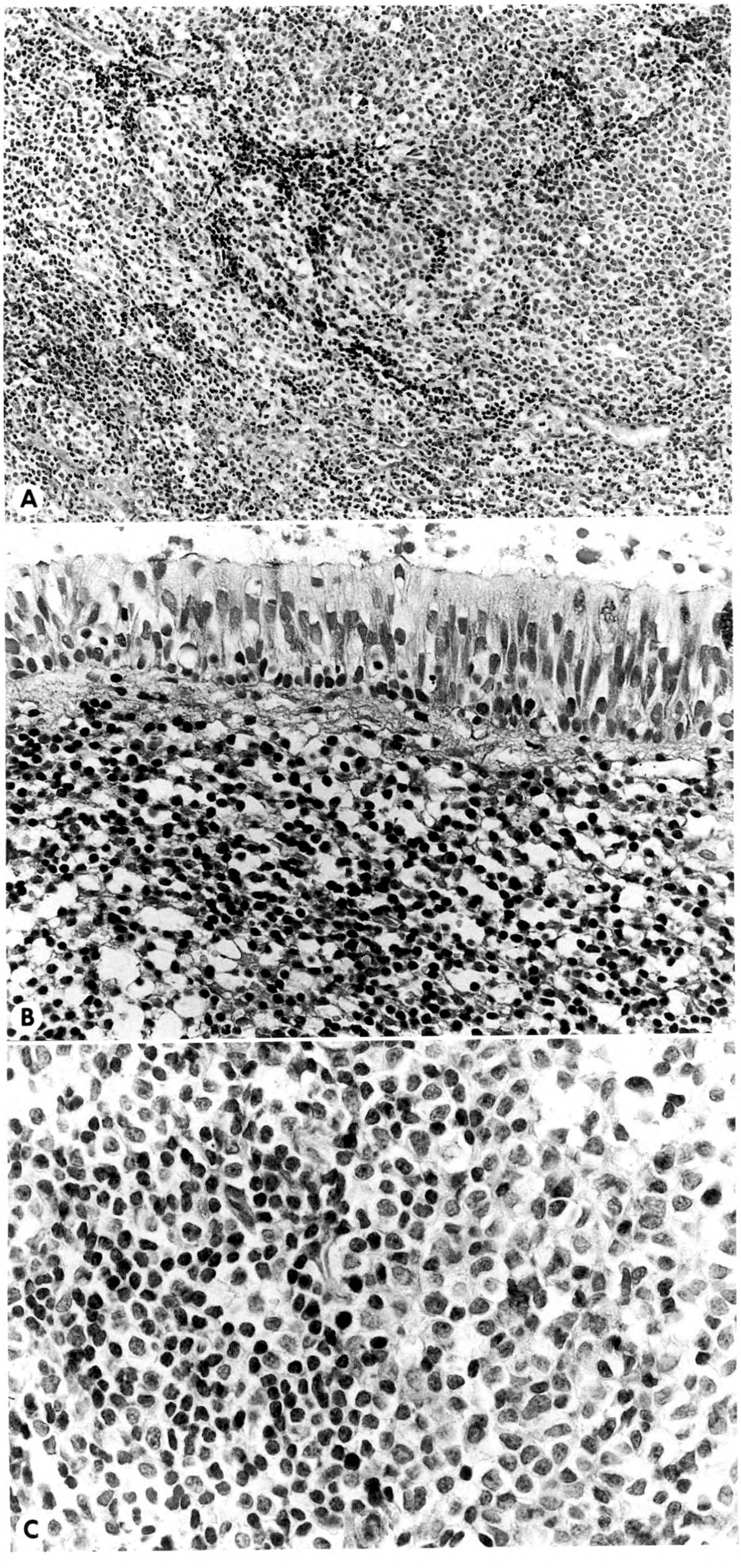
PRIMARY LARYNGEAL LYMPHOMA AND MALT LYMPHOMA CONCEPT

Any retrospective evaluation of the histopathologic features of reported cases of primary laryngeal lymphoma is impeded by the fact that various, fundamentally different classification systems for malignant lymphoma have been used and some terms, such as lymphosarcoma or reticulum cell sarcoma, used in older case reports, are now obsolete. The terminological problems are exacerbated by the fact that even now there is no widely accepted classification system for extranodal lymphoma. Moreover, the exact subtyping of lymphoid neoplasms by immunohistochemical methods came into general usage only in the mid-1980s, when antibodies for use on routinely processed biopsy specimens became available.

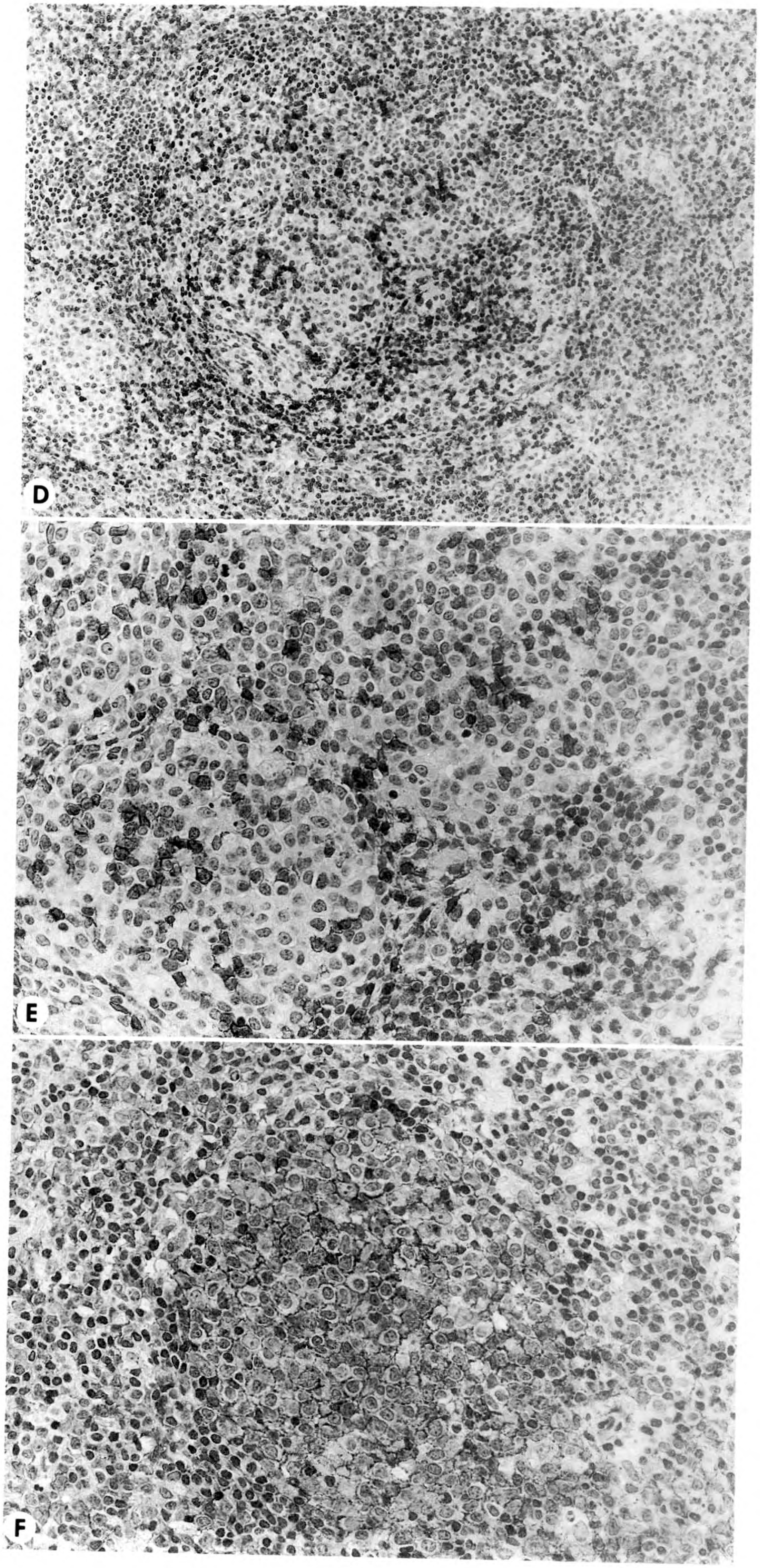
Our retrospective analysis, which must be regarded in the light of these limitations, has shown that laryngeal high-grade lymphomas are twice as common as those of low-grade malignancy and that B-cell tumors are far more common than T-cell tumors, with a ratio of about 6:1.[15]

Most of the recently published cases of high-grade primary laryngeal lymphoma were classified either as centroblastic or as immunoblastic lymphoma[17,18] on the criteria of the Updated Kiel Classification. A considerable proportion of the tumors of low-grade malignancy were classified as immunocytomas. The rarely reported primary laryngeal lymphomas of T-cell origin were subtyped as mycosis fungoides or as polymorphic reticulosis.[19-22]

Only 3 cases of laryngeal MALT-type low-grade lymphoma of the larynx have been reported.[2,23,24] In the case reported by Hisashi et al,[24] primary laryngeal malignant lymphoma coexisted with a squamous cell carcinoma. Isaacson and Norton[2] described a multifocal low-grade B-cell lymphoma of the upper aerodigestive tract with involvement of the nose and larynx. Although in the case of Diebold et al,[23] a diagnosis of primary laryngeal lymphoplasmacytic lymphoma (which, by definition, is a nodal-type lymphoma) was established, the tumor exhibited histopathologic features typical of MALT lymphoma, such as predominant proliferation of centrocyte-like cells, lymphoepithelial lesions, and reactive follicles with germinal centers. Thus, Diebold et al[23] themselves considered this tumor as a MALT lymphoma. We have recently seen a multicentric MALT lymphoma of the upper aerodigestive tract with involvement of Waldeyer's ring and the larynx (see Figure). A further case report of a multifocal low-grade lymphoma of the upper aerodigestive tract involving the larynx, soft palate, and nasal cavity contains only a very brief histologic description of the tumor, which



Multicentric low-grade B-cell lymphoma (MALT lymphoma) of upper aerodigestive tract with involvement of larynx. A) There is diffuse infiltration of connective tissue of left false vocal cord by lymphoid cells (periodic acid–Schiff [PAS], original ×140). Note pseudofollicular growth pattern of tumor. B) Intact respiratory columnar epithelium is seen at top of illustration (PAS, original ×350). There are dense aggregates of lymphoid cells in subepithelial connective tissue. Note that tumor cells do not exhibit epitheliotropism. C) At higher magnification, two types of lymphoid cells can be clearly discriminated: on left, small lymphocytes with round nuclei containing dense chromatin, and on right, medium-size lymphocytes with ovoid or irregularly contoured nuclei with little chromatin (PAS, original ×560). Note small nucleoli in considerable proportion of medium-size lymphocytes. (Continued on next page.)



(Continued) D) Immunohistochemical staining also demonstrates pseudofollicular growth pattern of lymphoma, in that most of lymphocytes surrounding pseudofollicular structures (consisting mainly of nonreactive, medium-size lymphocytes) are stained by T-cell marker ßF1 and therefore apparently belong to nonneoplastic cell clone (ßF1, avidin-biotin peroxidase complex [ABC] method, original ×140). E) At higher magnification it is clear that medium-size lymphocytes are not reactive with ßF1, but most of loosely scattered small lymphocytes are stained (ßF1, ABC, original ×350). F) Annular, ie, specific, staining of medium-size lymphocytes by B-cell–associated monoclonal antibody L26 is seen (L26/CD20, ABC, original ×350).

was definitively subtyped by the authors as a nodal-type low-grade lymphoplasmacytic lymphoma, so its classification as a MALT lymphoma does not seem justified.[25]

The larynx may contain diffuse and follicular lymphoid tissue even in healthy subjects. This is located mainly in the supraglottic portion and in the laryngeal ventricle, and is sometimes referred to as laryngeal tonsil.[26] MacLennan and Schofield[27] recently suggested that primary laryngeal lymphomas probably arise from this lymphoid tissue.

Laryngeal MALT lymphoma (like other laryngeal lymphomas) is usually confined to the sites of origin at diagnosis, although in some cases there is also involvement of adjacent lymph nodes (stage IE or IIE); its prognosis is consequently better than that of nodal low-grade lymphoma. However, there is no general agreement as to the most suitable treatment for laryngeal lymphoma.

We feel that the following conclusions can be drawn.

1. Because most of the reported cases of primary laryngeal lymphoma were diagnosed as being of high-grade malignancy and apparently contained no areas of low-grade lymphoma, it is impossible to establish whether these tumors were of MALT origin.

2. Primary MALT lymphoma of the larynx should always be considered in tumors with histopathologic features of low-grade B-cell lymphoma, or so-called pseudolymphoma.

3. Tumors evolving from MALT are rare among lymphomas of the upper aerodigestive tract in general, but a few cases of laryngeal lymphomas with the characteristic histopathologic features of MALT-type tumors have been reported.

REFERENCES

1. Freeman C, Berg JW, Cutler SJ. Occurrence and prognosis of extranodal lymphomas. Cancer 1972;29:252-60.

2. Isaacson PG, Norton AJ. Extranodal lymphomas. Edinburgh, Scotland: Churchill Livingstone, 1994.

3. Isaacson PG, Spencer J. Malignant lymphoma of mucosa-associated lymphoid tissue. Histopathology 1987;11:445-62.

4. Harris NL. Extranodal lymphoid infiltrates and mucosa-associated lymphoid tissue (MALT). A unifying concept. Am J Surg Pathol 1991;15:879-84.

5. Lennert K, Feller AC. Histopathology of non-Hodgkin's lymphoma (based on the Updated Kiel Classification). 2nd ed. New York, NY: Springer Verlag, 1992:13-52.

6. Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. Blood 1994;84:1361-92.

7. Gerard-Marchant R, Hamlin I, Lennert K, Rilke F, Stansfield AG, VanUnnik JAM. Classification of non-Hodgkin's lymphomas. Lancet 1974;ii:406-8.

8. Lennert K, Mohri N, Stein H, Kaiserling E. The histopathology of malignant lymphoma. Br J Haematol 1975;31(suppl):193-203.

9. Isaacson PG, Wright DH. Malignant lymphoma of mucosa-associated lymphoid tissue. A distinctive type of B-cell lymphoma. Cancer 1983;52:1410-6.

10. Isaacson PG. Lymphomas of mucosa-associated lymphoid tissue. Histopathology 1990;16:617-9.

11. DeVita S, Ferracioli G, DeRe V, et al. The polymerase chain reaction detects B cell clonalities in patients with Sjögren's syndrome and suspected malignant lymphoma. J Rheumatol 1994;21:1497-501.

12. Chan JKC, Ng CS, Isaacson PG. Relationship between high-grade lymphoma and low-grade B-cell mucosa-associated lymphoid tissue lymphoma (MALToma) of the stomach. Am J Pathol 1990;136:1153-64.

13. Fellbaum C, Hansmann M-L, Lennert K. Malignant lymphomas of the nasal cavity and paranasal sinuses. Virchows Arch [A] 1989;414:399-405.

14. Werner AJ, Lippert BM, Schmidt D, Rudert H. Subglottische Metastase eines multiplen Myeloms. Fallbericht und Literaturübersicht zu laryngealen Plasmocytomen. HNO 1991;39:405-9.

15. Horny H-P, Kaiserling E. Involvement of the larynx by hemopoietic neoplasms. An investigation of autopsy cases and review of the literature. Pathol Res Pract 1995;191:130-8.

16. Ferlito A, Doglioni C, Bontempini L, Arrigoni A. Metachronous coexistence of laryngeal pseudolymphoma and squamous cell carcinoma. ORL J Otorhinolaryngol Relat Spec 1984;46:202-9.

17. Morgan K, MacLennan KA, Narula A, Bradley PJ, Morgan AL. Non-Hodgkin's lymphoma of the larynx (stage IE). Cancer 1989;64:1123-7.

18. Ferlito A, Carbone A, Volpe R. Diagnosis and assessment of non-Hodgkin's malignant lymphomas of the larynx. ORL J Otorhinolaryngol Relat Spec 1981;43:61-78.

19. Hood AF, Mark GJ, Hunt JV. Laryngeal mycosis fungoides. Cancer 1979;43:1527-32.

20. Agarwal MK, Gupta S, Gupta OP. Mycosis fungoides of the larynx. Asian Med J 1982;25:180-4.

21. Nakashima T, Inamitsu M, Uemura T, Sugimoto T. Immunopathology of polymorphic reticulosis of the larynx. J Laryngol Otol 1989;103:955-60.

22. Ho FCS, Choy D, Loke SL, et al. Polymorphic reticulosis and conventional lymphomas of the nose and upper aerodigestive tract: a clinicopathologic study of 70 cases, and immunophenotypic studies of 16 cases. Hum Pathol 1990;21:1041-50.

23. Diebold J, Audouin J, Viry B, Ghandour C, Betti P, d'Ornano G. Primary lymphoplasmacytic lymphoma of the larynx: a rare localization of MALT-type lymphoma. Ann Otol Rhinol Laryngol 1990;99:577-80.

24. Hisashi K, Komune S, Inoue H, Komiyama S, Sugimoto T, Miyoshi M. Coexistence of MALT-type lymphoma and squa-

mous cell carcinoma of the larynx. J Laryngol Otol 1994;108: 995-7.

25. Bickerton RC, Brockbank MJ. Lymphoplasmacytic lymphoma of the larynx, soft palate and nasal cavity. J Laryngol Otol 1988;102:468-70.

26. Eggston AA, Wolff O. Histopathology of the ear, nose and throat. Baltimore, Md: Williams & Wilkins, 1947:845-65.

27. MacLennan KA, Schofield JB. Haemopoietic neoplasms. In: Ferlito A, ed. Neoplasms of the larynx. Edinburgh, Scotland: Churchill Livingstone, 1993:327-36.

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LETTERS TO THE EDITOR

To the Editor:

I was interested in the recent article by Maschka et al (*Ann Otol Rhinol Laryngol* 1996;105:70-1), who reported a case of prostatic cancer metastatic to the orbit and ethmoid sinus. The authors stated that only two cases of prostatic metastases to the paranasal sinuses have been previously reported in the literature: one in the ethmoid and one in the maxillary sinus. However, I would like to add that we reported a case of prostatic metastases involving all paranasal sinuses in 1993 and reviewed the previous literature, which showed seven other cases.[1]

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REFERENCE

1. Saleh HA, O'Flynn P, Jones NS. Prostatic metastases in the nose and paranasal sinuses. J Laryngol Otol 1993;107:629-32.

To the Editor:

I am writing concerning the paper of Drs Batsakis and El-Naggar, "Papillary Neoplasms (Heffner's Tumors) of the Endolymphatic Sac" (*Ann Otol Rhinol Laryngol* 1993;102:648-51). I have already communicated with Dr Batsakis to inform him and his coauthor that we were the first to postulate, in 1985, the endolymphatic sac origin of a papillary tumor in a petrous bone (of a 36-year-old man).[1] We came to this conclusion after detailed light and electron microscopic and immunohistochemical studies of the normal human endolymphatic sac from autopsies, study of the epithelium of this man's tumor, and studies of a choroid plexus papilloma and papillary thyroid carcinoma that were considered in the differential diagnosis.

Like Dr Heffner, who wrote extensively about these tumors 4 years later in 1989,[2] we derived our hypothesis from firsthand knowledge of a small papillary adenoma in the endolymphatic sac that was serendipitously discovered by Hassard et al,[3] during a decompression procedure for Meniere's disease, and reported in 1984. Dr Hassard, a practicing ear surgeon, was a former colleague of ours in Halifax, and I was the neuropathologist to whom this lesion of the endolymphatic sac was first shown. While it resembled a choroid plexus papilloma, we realized that Dr Hassard and his collegues had encountered a tumor in the endolymphatic sac that had not been previously recognized. This lesion was also referred to the Armed Forces Institute of Pathology and was an index case in Dr Heffner's 1989 paper.[2] I trust your journal will consider these facts in their proper perspective.

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REFERENCES

1. MacDougall AD, Sangalang VE, Huestis S. A previously unrecognized papillary endolymphatic sac tumor presenting as a cerebellopontine angle lesion [Abstract]. Can J Neurol Sci 1985;12:200-1.

2. Heffner DK. Low-grade adenocarcinoma of probable endolymphatic sac origin. A clinicopathologic study of 20 cases. Cancer 1989; 64:2292-302.

3. Hassard AD, Boudreau SF, Cron CC. Adenoma of the endolymphatic sac. J Otolaryngol 1984;13:213-6.

BOOK REVIEW

Hereditary Hearing Loss and Its Syndromes

Robert J. Gorlin, Helga V. Toriello, and M. Michael Cohen, Jr. Hard cover, illustrated, indexed, 488 pages, 1995. Oxford University Press, New York, NY, $195.

This volume is a sequel to the 1976 text by Konigsmark and Gorlin, *Genetic and Metabolic Deafness*. The general format is the same. That is to say, the various forms of hereditary hearing losses are considered, then syndromal deafness is classified according to structure as external ear, eye, kidney, nervous system, endocrine disease, chromosomal disorders, integumentary disorders, oral and dental disorders, and miscellaneous disorders.

Included in the present volume is a brief history of genetic hearing loss, an approach to the process of syndrome delineation, epidemiology, etiology, genetic patterns, and embryology of the ear.

It is estimated that new syndromes are being described at a rate of one or more per week, although some represent variable expressions of previously reported conditions. There are newly recognized conditions. The reason for these new discoveries is the advances in human cytogenetics, which has passed through various stages since the 1950s. This is well conveyed in the opening chapters of the book. Stage 1, from 1956 to the 1960s, detected mostly abnormalities of chromosome number and few structural aberrations. In 1970, stage 2 consisted of banding techniques that led to a large number of interstitial and terminal deletions and duplications, double deletion, deletion duplication, and double duplication. The third stage was introduced in the 1980s and included prometaphase staining and combining cyanogenic and molecular methods for identifying microdeletions and for gene mapping.

In fact, it is said that so much progress has been made in genetics that now it is easier to identify a hereditary hearing loss. That is true when a syndrome can be identified; then it becomes easy. Twenty-three types of hereditary hearing loss with no associated abnormalities were considered in chapter 5. In such cases I still find it difficult to diagnose hereditary hearing loss and especially to differentiate hereditary loss from that of congenital viral origin (when, as is typical, proper viral urine cultures are not done at birth).

Also, in chapter 5, there are statements that need clarification such as follows. How does vestibular testing differentiate autosomal dominance from recessive hearing loss? Too, there is not a clear-cut definition to differentiate congenital from acquired hearing loss. Midfrequency sensorineural hearing loss is caused by prenatal rubella, ototoxins, or noise trauma. Is this true? The definition of Mondini dysplasia is characterized by 1½ turns of bony cochlea rather than the usual 2½ turns. Why can't it be any variation from the normal 2½ turns? Such questions do not detract from this very scientific, outstanding, comprehensive book, which will facilitate the differential diagnosis for the clinician, as well as enhance the knowledge of clinical signs of the various syndromes, their hereditary and biochemical background, and their enzymatic deficiencies.

This book is recommended as a reference for every otolaryngologist, along with paramedical supporters of the specialty.

DENNIS G. PAPPAS, MD
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ANNALS OF OTOLOGY, RHINOLOGY & LARYNGOLOGY

VOLUME 105 AUGUST 1996 NUMBER 8

CONTENTS

FROM THE AMERICAN BRONCHO-ESOPHAGOLOGICAL ASSOCIATION

Effect of Stenting on Graft Vascularization After Laryngotracheoplasty
Thomas C. Logan, MD; Douglas E. Henrich, MD; William W. Shockley, MD 585

FROM THE AMERICAN LARYNGOLOGICAL ASSOCIATION

Long-Term Follow-up of Recurrent Laryngeal Nerve Avulsion for the Treatment of Spastic Dysphonia
Donald T. Weed, MD; Brian S. Jewett, MD; Cheryl Rainey, MS;
David L. Zealear, PhD; R. E. Stone, PhD;
Robert H. Ossoff, DMD, MD; James L. Netterville, MD 592

Autologous Fat Injection for Unilateral Vocal Fold Paralysis
Maisie L. Shindo, MD; Lauren S. Zaretsky, MD; Dale H. Rice, MD 602

INDEPENDENT CONTRIBUTIONS

Treatment of Chronic Paranasal Sinus Pain With Minimal Sinus Disease
Martin A. Acquadro, MD, DMD; William W. Montgomery, MD 607

Residue of Middle Ear Cholesteatoma After Intact Canal Wall Tympanoplasty: Surgical Findings at One Year
Kiyofumi Gyo, MD; Yasushi Sasaki, MD;
Yasuyuki Hinohira, MD; Naoaki Yanagihara, MD 615

Endonasal Endoscopic Closure of Cerebrospinal Fluid Fistulas at the Anterior Cranial Base
Mislav Gjuric, MD, DSc; Ulrich Goede, MD;
Henning Keimer; Malte Erik Wigand, MD 620

Microbiology of Tracheal Granulation Tissue Associated With Silicone Airway Prostheses
Mark T. Brown, MD; William W. Montgomery, MD 624

HLA Class II–Associated Genetic Susceptibility in Idiopathic Progressive Sensorineural Hearing Loss
Ming-Yu Cao, MD; Joëlle Thonnard, MD; Naïma Deggouj, MD;
Michel Gersdorff, MD; Marianne Philippe, MD;
Jean-Claude Osselaer, MD; Jean-Paul Tomasi, MD 628

Circulating Intercellular Adhesion Molecule–1 and Its Cellular Expression in Head and Neck Non-Hodgkin's Lymphomas, Including Lethal Midline Granuloma
Yasuaki Harabuchi, MD, PhD; Akikatsu Kataura, MD, PhD; Kohzoh Imai, MD, PhD....634

Secretory Otitis Media in Adults: I. The Role of Mastoid Pneumatization as a Risk Factor
Jacob Sadé, MD; Camil Fuchs, PhD 643

Role of Substance P in the Vascular Response of Nasal Mucosa in Nasal Allergy
Akiyoshi Konno, MD; Tsutomu Numata, MD; Nobuhisa Terada, MD;
Toyoyuki Hanazawa, MD; Hiroshi Nagata, MD; Hideaki Motosugi, MD 648

Otoneurological Expert System
Erna Kentala, MB; Ilmari Pyykkö, MD; Yrjö Auramo, PhM;
Martti Juhola, PhD 654

High-Resolution Computed Tomographic Evaluation of the Cochlear Capsule in Otosclerosis: Relationship Between Densitometry and Sensorineural Hearing Loss
E. Alpin Güneri, MD; Emel Ada, MD; Kerim Ceryan, MD;
Ataman Güneri, MD 659

SOFTWARE REVIEW

Adult Airway Management Principles and Techniques
Keith F. Clark, MD 665

IMAGING CASE STUDIES OF THE MONTH

Metastatic Breast Carcinoma Presenting as Cavernous Sinus Syndrome
Matthew W. Ryan, MD; Christopher H. Rassekh, MD; Gregory Chaljub, MD 666

Hypopharyngeal Dilatation in a Musician
William J. Garvis, MD; Henry T. Hoffman, MD 669

PATHOLOGY CONSULTATION

Pharyngeal Hypophysis
Gregory N. Fuller, MD, PhD; John G. Batsakis, MD 671

MISCELLANEOUS

Instructions to Authors 5a

Information for Subscribers 2a

Index to Advertisers 8a

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EFFECT OF STENTING ON GRAFT VASCULARIZATION AFTER LARYNGOTRACHEOPLASTY

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The management of pediatric airway stenosis has been an important topic of debate over the last few decades as prolonged intubation has become more common in the neonate. Although many surgical options are available, most would agree that use of expansion techniques (laryngotracheoplasty) with cartilaginous grafting is the procedure of choice for the severely stenotic airway. Controversy persists, however, regarding the role of stents. Advocates feel that stents serve to counteract scar contracture and support the newly constructed airway. In contrast, recent studies suggest that stenting results in impaired wound healing and an increased complication rate. The present study addresses the effect of stenting on the vascularization of cartilaginous grafts in an animal model. Thirty-six New Zealand white rabbits were evaluated after laryngotracheoplasty with autogenous cartilage grafting. Half of the animals were stented with a 2-cm section of an endotracheal tube that was secured just below the glottis. Three rabbits from each group were then painlessly sacrificed on days 4, 6, 8, 10, 14, and 21. Gross and histologic comparisons of the stented and nonstented specimens revealed similar wound healing. Measurements of graft vascularization were obtained with a computerized image measurement program, and a comparison was made regarding the rate of vascularization. There was a statistically significant increase in the rate of vascularization in the stented group (mean 73% versus 55% at day 10; $p < .05$). This analysis suggests that stenting does not inhibit early wound healing and specifically graft vascularization after laryngotracheoplasty.

KEY WORDS — graft vascularization, laryngotracheoplasty, stent, subglottic stenosis, wound healing.

INTRODUCTION

The incidence of acquired subglottic stenosis has risen significantly over the past three decades, as prolonged endotracheal intubation has become the standard of ventilatory support in the neonate and infant. Recent literature suggests an incidence of 2.5% in neonates subjected to long-term intubation.[1] Most authorities agree that an expansion technique (laryngotracheoplasty) with cartilaginous grafting provides the best opportunity for correction of the severely stenotic airway. However, clinical results of this technique are unpredictable, as indicated by the reported decannulation rates of 60% to 92%.[2-4]

Postoperative stenting of the reconstructed airway has been implicated as a source of poor wound healing and graft failure. Olson[5] has stated that stents are best avoided in the reconstructed airway, and a number of experimental studies have supported this view. In one of the first studies, Thomas and Stevens[6] evaluated airway healing after controlled laryngeal injury in dogs and found that the nonstented group had less stenosis and decreased granulation tissue compared to three other groups stented with various stenting materials. Albert et al[7] concluded that an increase in duration of stenting in the undamaged rabbit trachea was associated with increased inflammation. Wiatrak et al[8] investigated the effect of stenting on the reconstructed airway using a goat model and found that there was increased inflammation and decreased epithelialization in the stented group.

An important component of wound healing and graft survival that has not been examined in regard to the effects of stenting is the rate of revascularization of the cartilage graft. Cotton[9] suggested that the increased neochondrification of posterior laryngeal grafts in his rabbit study might be attributable to the more vascular nature of the posterior airway. In another animal study of airway reconstruction, Shockley[10] stated that revascularization of cartilage grafts was essential to successful reconstruction. Thus, the effects of stenting on vascular ingrowth to a newly placed graft may have a significant impact on graft survival and postoperative success. The present study was designed to address this question using a rabbit model of laryngotracheoplasty and cartilage grafting.

MATERIALS AND METHODS

Forty juvenile New Zealand white rabbits (13 to 15

From the Division of Otolaryngology–Head and Neck Surgery, University of North Carolina, Chapel Hill, North Carolina. This study was performed in accordance with the PHS Policy on Humane Care and Use of Laboratory Animals, the NIH *Guide for the Care and Use of Laboratory Animals*, and the Animal Welfare Act (7 U.S.C. et seq.); the animal use protocol was approved by the Institutional Animal Care and Use Committee (IACUC) of the University of North Carolina.

Presented at the meeting of the American Broncho-Esophagological Association, Palm Desert, California, May 2-4, 1995. Winner of Resident Essay Award.

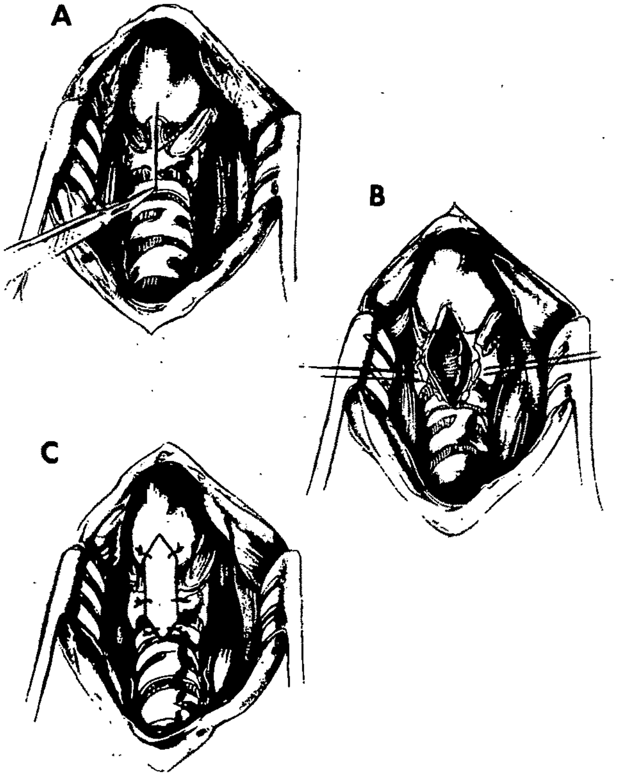

Fig 1. Experimental protocol. A) Midline division of lower third of thyroid cartilage, cricoid cartilage, and first tracheal ring. B) Retraction of cut margins of airway. C) Auricular cartilage graft sewn to anterior airway defect.

weeks of age) underwent identical laryngotracheoplasty procedures and postoperative care as described below. The study protocol was approved by an Internal Animal Care and Use Committee prior to initiation. Each animal was administered an intramuscular injection of ketamine hydrochloride 30 mg/kg and xylazine hydrochloride 6 mg/kg for general anesthesia. All animals were allowed to breathe spontaneously without the use of an artificial airway. in addition, each animal was given an intramuscular injection of penicillin G 60,000 units/kg. The neck and the dorsal aspect of the right ear were shaved. The right ear was prepared with povidone-iodine and alcohol and draped with sterile towels for graft harvesting. A skin incision was made at the base of the ear, and a 25 × 10-mm section of auricular cartilage was harvested. Care was taken to preserve the perichondrium on at least one side of the graft. The cartilage was immediately cut into a 17 × 5-mm boat-shaped graft by means of a standardized template and then placed in a saline-soaked gauze to keep moist prior to placement. The skin incision was closed with continuous 4-0 Prolene, and Bacitracin Zinc Ointment was applied to the wound.

The rabbit was then placed supine with the ears tucked and the neck extended. The surgical field was prepared and draped in a sterile fashion. With a scalpel, a midline ventral neck incision was made and carried down to the strap muscles. The strap muscles were bluntly separated in the midline and retracted laterally to expose the trachea. The lateral paratracheal tissues were left undisturbed to prevent disruption of the tracheal blood supply. An anterior laryngofissure was performed. The incision extended from the lower third of the thyroid cartilage (just below the glottis) through the cricoid cartilage and first tracheal ring (Fig 1A). One half of the animals were randomly assigned to receive an intraluminal stent. A 2-cm portion of a polyvinyl endotracheal tube (outside diameter 4.8 mm) was secured to the lower portion of the thyroid cartilage with a through-and-through suture of 4-0 polypropylene. The sutures were secured to the strap muscles on each side. The stent did not extend through the level of the true vocal cords.

The cartilage graft was placed into the laryngofissure, with the preserved perichondrium on the intraluminal surface. Six 6-0 polypropylene sutures were used to secure the graft to the inferior margin of the thyroid cartilage, the cricoid cartilage, and the first tracheal ring (Fig 1C). The strap muscles were reapproximated in the midline with continuous 4-0 polyglactin suture, and the skin edges were closed with continuous 4-0 polypropylene suture. An antibiotic ointment was applied. All rabbits were housed in approved animal facilities and fed a standard diet of rabbit chow and water.

Three animals from the stented group and 3 from the control group were painlessly sacrificed on days 4, 6, 8, 10, 14, and 21. An intramuscular injection of ketamine hydrochloride 30 mg/kg and xylazine hydrochloride 6 mg/kg was administered for general anesthesia. By aseptic technique, the abdomen was entered and the abdominal aorta was cannulated to the aortic arch with a polyethylene catheter. Approximately 10 mL of India ink (Hunt brand) was instilled slowly through the catheter to allow staining of the vasculature of the head and neck. Adequate staining was determined by involvement of the oral mucosa. The rabbit was immediately sacrificed with an intramuscular overdose of ketamine and xylazine. The airway was then harvested and placed in a 10% paraformaldehyde buffered solution. Stents were removed at the time of sacrifice to allow for adequate fixation of the tissues adjacent to the stent.

Graft vascularization measurements were obtained by the primary author (T.C.L.) in a blinded fashion. Each airway was divided posteriorly and retracted to reveal the intraluminal portion of the graft. The airway was visualized with epi-illumination using a Wild stereo-dissecting microscope. A video camera

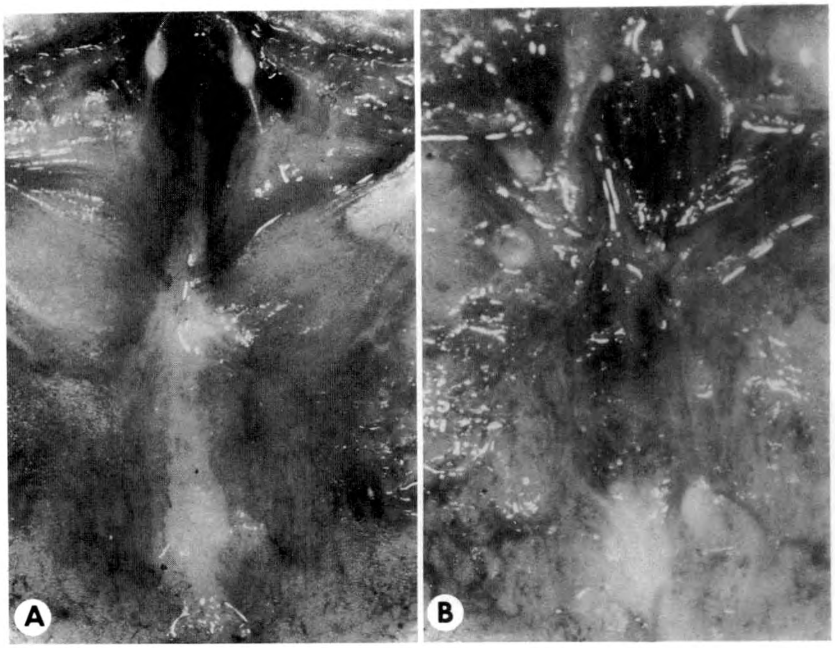

Fig 2. Photomicrographs of intraluminal views of representative cartilage grafts on postoperative day 10. A) From nonstented rabbit (48% vascularized). B) From stented rabbit (71% vascularized). Note organized vascular markings and increased area of graft vascularized in stented group.

(MTI model CCD 72S) was attached to the microscope for digital video image analysis of the graft. The image was captured, digitized, and stored in the memory board (FG100-AT, Series 100; Imaging Technology) within an IBM AT computer. The digitized image was then labeled by number only and uncoded after two individual measurements had been performed. The area of graft devoid of vascular supply was outlined with a mouse-driven cursor and calculated by a software program (ImageMeasure; Microscience). The percentage of graft vascularization was calculated by subtracting the area of graft not vascularized from a previously determined value of total graft area.

Histologic preparation of the tissues was performed after all measurements were obtained and photodocumentation had been completed. The tissues were dehydrated with graded ethanol washes (30%, 50%, 75%, 95%, and 100%) followed by two xylene washes. The tissues were embedded in paraffin and serially sectioned 8 μm thick by means of a microtome. The sections were mounted on glass slides, stained with hematoxylin and eosin, and covered.

The prepared tissues were submitted in a blinded fashion to a board-certified pathologist for histologic analysis and comparison. Specific criteria for comparison included the character of the epithelium, the presence or absence of squamous metaplasia, the presence or absence of cilia, the character of inflammation, and the presence or absence of cartilage necrosis and/or ulceration.

RESULTS

A total of 40 rabbits underwent laryngotracheoplasty. Four were excluded from analysis because of postoperative complications. One rabbit died of an anesthetic overdose, another of pneumonia. Two rabbits (1 stented and 1 nonstented) had no evidence of complications until the time of sacrifice, at which time they were noted to have purulence in the soft tissues of the neck with graft dehiscence. Neither developed respiratory distress. The remaining 36 rabbits constituted the study group. Relatively common postoperative findings included decreased oral intake on the first postoperative day and mild upper airway noise (more common in the stented group) for the first several postoperative days.

Three animals from both the stented group and the nonstented group were sacrificed on postoperative days 4, 6, 8, 10, 14, and 21. Clinical observations of the dissected specimens, statistical analysis of vascularization, and histologic analysis are described below.

Clinical Observations. Tracheal specimens from animals sacrificed on postoperative day 4 were characterized by bridging of the step-off between the cut surface of the tracheal lumen and the graft. Initially there appeared to be a disorganized accumulation of mucosa and underlying stroma from which the regenerating epithelium arose. In specimens from animals sacrificed on postoperative days 6 through 10, a leading edge of small capillaries trailed the epithelial border, extending toward the center of the graft (Fig 2). The regenerated epithelium and vascularization first met in the central area of the graft. The remaining superior and inferior areas of graft barren of vascularization filled in at various rates.

The vascular organization of the newly placed

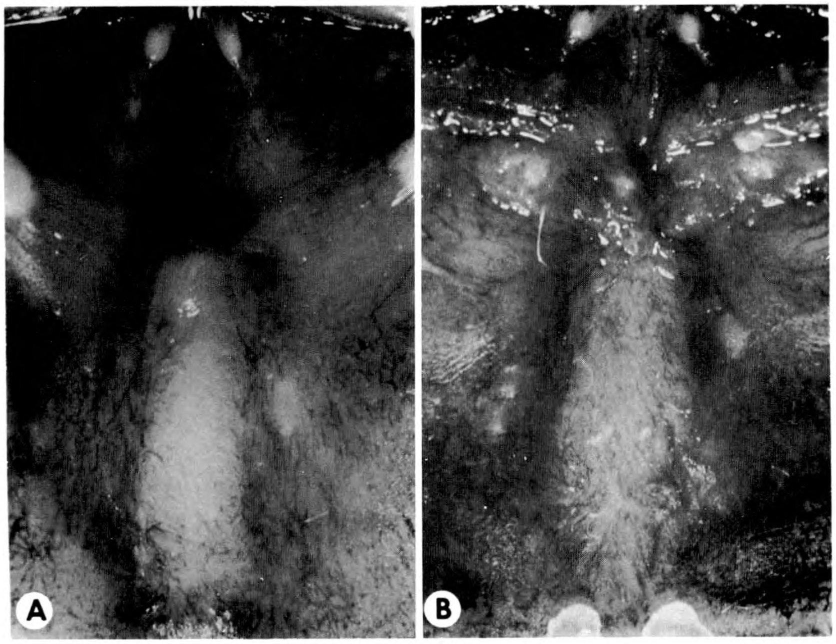

Fig 3. Photomicrographs of intraluminal views of representative cartilage grafts on postoperative day 21. A) From nonstented rabbit. B) From stented rabbit. Both grafts are completely epithelialized and vascularized, but stented specimen is characterized by hypervascularity and decreased vascular organization.

graft was characteristically different between the two groups. The native laryngeal mucosa surrounding the graft supplied the origin of epithelium with its blood supply. The stented group displayed an increase in the number of vessels within this surrounding area as compared to the control group (Fig 3). This difference remained consistent at each day of sacrifice. In addition, as the graft became completely vascularized, the multitude of small capillary channels that traversed the graft responded differently. In the control group there was a decrease in vascular density, leaving a somewhat organized pattern of vascularity (Fig 3A). The stented group, however, showed a persistence of this hypervascularity, with less reorganization (Fig 3B).

Statistical Analysis. The statistical analysis for percent graft vascularization focused primarily on the data from days 6, 8, and 10. The 4-day group revealed minimal vascular ingrowth in both the stented and control animals. By day 14 all grafts had been completely vascularized. The comparisons between stented and nonstented groups and among days 6, 8, and 10 were based on a corresponding two-way analysis of variance (ANOVA) statistical model. The use of this method was justified through an expanded model that indicated that the difference between the stented and nonstented groups at any given day was consistent across each of the study days ($p > .25$). Thus, the model applying this assumption was supported.

When this ANOVA model applied the components for day and percent graft vascularization of each group, there was a statistically significant difference between the stented and nonstented groups. Figure 4A shows the average percentage of graft vascularization of each group with respect to time. Figure 4B is a scattergram of the percentage of graft vascularization for each animal with respect to time. The 4-day groups showed limited vascularization and were basically identical in graft vascularization. However, a comparison of the 6-, 8-, and 10-day groups showed an increase in vascularization of the

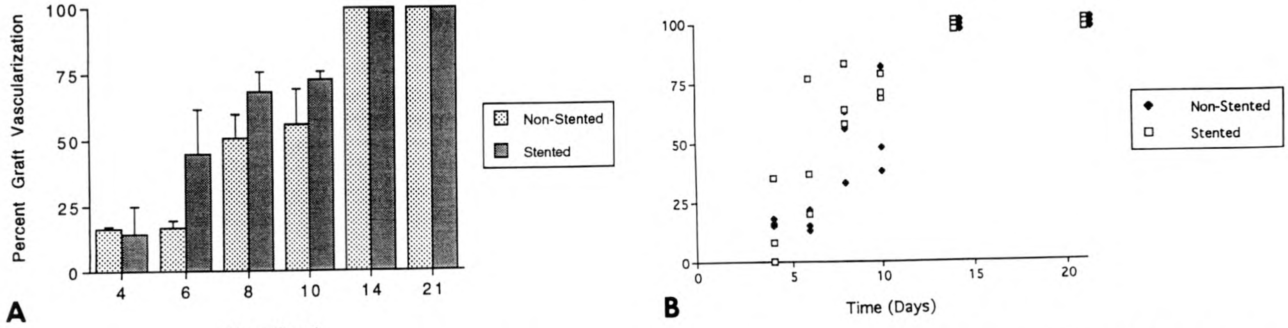

Fig 4. A) Average cartilage graft vascularization with respect to day of sacrifice. Bars show standard deviation. B) Scatter plot of cartilage graft vascularization with respect to day of sacrifice.

stented rabbits as compared to the controls ($p < .05$). There was an average 18% increase in vascularization at each of these days. All grafts were completely vascularized by day 14 and remained vascularized through day 21.

Histologic Analysis. Histologic analysis revealed similar early wound healing characteristics in the stented and nonstented groups. There was an early influx of inflammatory cells into all of the specimens with a predominance of polymorphonuclear leukocytes. The epithelium overlying the graft was initially nonciliated columnar; however, by day 10 there was a transition into pseudostratified epithelium, with occasional areas of cilia, identified in both groups. Squamous metaplasia occurred rarely but was identified in three grafts of the nonstented group. The previously noted trend toward increased neovascularization in the stented group was also noted on histologic comparison. Neochondrification was identified at the cut margins of many of the grafts. All underlying cartilage appeared to be viable, with no evidence of cartilage necrosis or fibrosis.

Granulation tissue was commonly encountered in the stented group; however, the graft and adjacent tissues were not involved. The two areas where granulation tissue was identified were the inferior aspects of the arytenoid mucosa (where the superior aspect of the stent came into contact) and the tracheal mucosa that was traumatized by the inferior aspect of the stent. These areas were characteristically denuded of epithelium, with variable amounts of fibroblasts and stromal constituents. None of the involved areas were thought to be infected, and no significant sequelae resulted.

DISCUSSION

As prolonged endotracheal intubation became commonplace for ventilatory support in the neonate, the occurrence of acquired subglottic stenosis increased significantly. In the 1970s the reported incidence following prolonged intubation in neonates ranged from 12% to 20%.[11,12] A refinement of management principles, including intubation techniques, endotracheal tube support, and physical characteristics of the endotracheal tube, led to a falling incidence. Despite these measures, however, subglottic stenosis continues to occur in a small number of patients. In a recent study from a large pediatric institution, an incidence of 2.5% was cited.[1]

The mechanism of subglottic injury has been extensively investigated. The precipitating insult is mucosal edema and ulceration.[13] This may be initiated by traumatic intubation, or may result from repeated mechanical trauma from the endotracheal tube. The relationship between the length of intubation and the degree of injury is poorly understood. Many authors believe that mucosal edema and ulceration progress in response to persistent trauma of the endotracheal tube and that the severity of outcome is related to the time period of intubation.[14,15] Multiple episodes of ulceration and repair result in excessive scar tissue formation. In addition, loss of normal ciliary flow with mucociliary stasis may lead to secondary infection. Squire et al[16] have shown a correlation between respiratory tract infection and the severity of experimentally induced tracheal stenosis.

Fairchild[17] reported the first tracheal reconstruction in 1927. Since that time, there have been many surgical procedures designed to address the stenotic airway, many of which utilize grafts and stents. The role of laryngotracheoplasty with autogenous cartilage grafting for the severely stenotic airway has become well established. Yet, intraluminal stenting to stabilize the implanted graft remains controversial. In theory, the stent serves to support the newly constructed airway, counteract scar contracture, and function as a scaffold for reepithelialization of the airway. Since the first description of laryngeal stenting by Schmiegelow[18] in 1929, numerous modifications of stenting techniques and materials have been proposed. In 1956 Rethi[19] described the use of a Mikulicz tampon for the initial 3 postoperative days after anterior and posterior laryngofissure, followed by placement of a long-term tracheal dilator that was secured to the tracheostomy cannula. In 1965 Montgomery[20] developed the silicone T-tube, and shortly thereafter Aboulker[21] designed a Teflon stent that could be fashioned to accommodate an indwelling tracheostomy tube. These modifications made long-term outpatient stenting feasible while focusing on a more inert material. Evans[22] advocated the use of a "Swiss rolled" silicone sheet that would unroll within the lumen to accommodate any size airway. Numerous articles support the advantages of stents.[23-26]

In contrast, many authors argue that stenting results in impaired wound healing and an increased complication rate.[5,6,8,27] Irritation of laryngeal and tracheal mucosa by the stent may result in impaired wound healing. Theoretically, repeated trauma to the cartilage graft and adjacent mucosa would prevent epithelialization and vascularization and would promote formation of granulation tissue. This may have a significant deleterious effect on the success of the newly placed autogenous graft.

This study demonstrated that stenting does not impede early wound healing or, specifically, graft vascularization after laryngotracheoplasty. In fact, the stented group showed a statistically significant increase in the rate of vascularization on postoperative days 6 through 10. Regardless of whether or not a stent was utilized, all cartilage grafts were completely vascularized by postoperative day 14 and remained vascularized through postoperative day 21. This suggests that stenting does not appear to delay graft vascularization and may actually accelerate its early stages. It is not possible from this study to extrapolate the long-term effects stenting may have on the cartilage graft and its blood supply.

Previous authors have suggested that granulation tissue is a significant problem that may be encountered with stenting. Our histologic observations support an increased presence of granulation tissue in stented animals, but calls into question the significance of these findings. It should be emphasized, however, that our model used a normal airway, not one affected by stenosis or fibrosis. The two areas of involvement were the inferior mucosal surface of the arytenoid cartilages and the tracheal mucosa at the distal aspect of the stent. This granulation tissue had no deleterious effects that could be determined during the study period and did not show a propensity for infection. The graft itself was never involved.

While early wound healing has been detailed, the long-term effects of stenting remain unclear. In addition, the appropriate duration of stenting in the postoperative period remains controversial. Finally, these procedures were performed on normal rabbit larynges, and the characteristic fibrotic tissues of a stenosed subglottis may react very differently after laryngotracheoplasty.

CONCLUSION

Subglottic stenosis is a significant complication of prolonged intubation in the neonate. Laryngotracheoplasty has become the standard of care for the severely stenotic airway and often requires the use of grafts and/or stents. This study contradicts previous reports that the use of stents is detrimental to early wound healing in the reconstructed airway. We found that the stented group had a comparable rate of vascularization postoperatively and had no evidence of an increased complication rate. In fact, there appeared to be a positive influence of stents on vascularization of the cartilage grafts. While granulation tissue did occur in the stented group, it did not appear to affect the postoperative course of the animal, nor did it impair graft vascularization. The clinical significance of the above findings requires further investigation.

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REFERENCES

1. Nicklaus PJ, Crysdale WS, Conley S. Acquired neonatal subglottic stenosis. Arch Otolaryngol Head Neck Surg 1990; 116:902.

2. Cotton RT, Gray SD, Miller RP. Update of the Cincinnati experience in pediatric laryngotracheal reconstruction. Laryngoscope 1989;99:1111-6.

3. Hof E. Surgical correction of laryngotracheal stenoses in children. Prog Pediatr Surg 1987;21:29-35.

4. Maddalozzo J, Holinger LD. Laryngotracheal reconstruction for subglottic stenosis in children. Ann Otol Rhinol Laryngol 1987;96:665-9.

5. Olson NR. Wound healing by primary intention in the larynx. Otolaryngol Clin North Am 1979;12:735-40.

6. Thomas GK, Stevens MH. Stenting in the experimental laryngeal injuries. Arch Otolaryngol 1975;101:217-21.

7. Albert DM, Cotton RT, Cohn P. The use of alcohol-stored cartilage in experimental laryngotracheal reconstruction. Int J Pediatr Otolaryngol 1989;12:735-40.

8. Wiatrak BJ, Albert DM, Holmes DK, Cotton RT. Cartilage graft epithelialization. A preliminary study using a goat model. Arch Otolaryngol Head Neck Surg 1993;119:777-81.

9. Cotton RT. The problem of pediatric laryngotracheal stenosis: a clinical and experimental study on the efficacy of autogenous cartilaginous grafts placed between the vertically divided halves of the posterior lamina of the cricoid cartilage. Laryngoscope 1991;101(suppl 56).

10. Shockley W. Airway reconstruction with cartilaginous grafts: a study of the early stages of healing. Laryngoscope (in press).

11. Fearon B, Ellis D. The management of long term airway problems in infants and children. Ann Otol Rhinol Laryngol 1971;80:669-77.

12. Gaudet PT, Peerless A, Sasaki CT. Pediatric tracheostomy and associated complications. Laryngoscope 1978;88:1633-41.

13. Hawkins DB. Hyaline membrane disease of the neonate prolonged intubation in management: effects on the larynx. Laryngoscope 1978;88:201-24.

14. Strong RM, Passy V. Endotracheal intubation. Complications in neonates. Arch Otolaryngol 1977;103:329-35.

15. Papsidero MJ, Pashley NRT. Acquired stenosis of the upper airway in neonates. An increasing problem. Ann Otol Rhinol Laryngol 1980;89:512-4.

16. Squire R, Brodsky L, Rossman J. The role of infection in the pathogenesis of acquired tracheal stenosis. Laryngoscope 1990;100:765-70.

17. Fairchild FR. Reconstruction of the trachea. Surg Gynecol Obstet 1927;44:119-22.

18. Schmiegelow E. Stenosis of the larynx: a new method of surgical treatment. Arch Otolaryngol 1929;9:473-93.

19. Rethi A. An operation for cicatricial stenosis of the larynx. J Laryngol Otol 1956;70:283-93.

20. Montgomery W. T-tube tracheal stent. Arch Otolaryngol 1965;82:320-1.

21. Aboulker P. Modifications apportées à l'intervention de Réthi. Intérêt dans les sténoses laryngo-trachéales et trachéales. Ann Otolaryngol 1966;83:98-106.

22. Evans JN. Laryngotracheoplasty. Otolaryngol Clin North Am 1977;10:119-23.

23. Cotton RT, Evans JNG. Laryngotracheal reconstruction in children. Five-year follow-up. Ann Otol Rhinol Laryngol 1981;90:516-20.

24. Grahne B. Operative treatment of severe chronic traumatic laryngeal stenosis in infants up to three years old. Acta Otolaryngol (Stockh) 1971;72:134-7.

25. Crysdale W. Subglottic stenosis in children. A management protocol plus surgical experience in 13 cases. Int J Pediatr Otorhinolaryngol 1983;6:23-36.

26. Maran AG, Geissler PR. A new material for laryngeal prosthesis. J Laryngol Otol 1970;84:1147-51.

27. Goode RL, Shinn JB. Long-term stenting in the treatment of subglottic stenosis. Ann Otol Rhinol Laryngol 1977;86:795-8.

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SUPRAESOPHAGEAL COMPLICATIONS OF REFLUX DISEASE

Supraesophageal Complications of Reflux Disease will be held November 21-23, 1996, in Tucson, Arizona. For further information, contact Reza Shaker, MD, MCW Dysphagia Institute, Division of Gastroenterology, Froedtert Memorial Lutheran Hospital, 9200 W Wisconsin Ave, Milwaukee, WI 53226; telephone (414) 259-2742; fax (414) 384-8480.

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PEDIATRIC OTORHINOLARYNGOLOGY

The Seventh International Congress of Pediatric Otorhinolaryngology will be held in Helsinki, Finland, June 7-10, 1998. For more information, contact Pekka Karma, MD, Chairman, or Hans Ramsay, MD, Secretary, ENT Department, Helsinki University Central Hospital, Haarmaninkatu 4 E, FIN-00290 Helsinki, Finland; telephone 358 9 4713019; fax 358 9 4715010; E-mail ramsay@hansram.pp.fi.

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LONG-TERM FOLLOW-UP OF RECURRENT LARYNGEAL NERVE AVULSION FOR THE TREATMENT OF SPASTIC DYSPHONIA

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Long-term follow-up of 3 to 7 years is reported on 18 patients who had undergone recurrent laryngeal nerve avulsion (RLNA) for the treatment of adductor spastic dysphonia (SD). Data on neural regrowth after previous recurrent laryngeal nerve section (RLNS) are presented in 2 of these 18 patients. We introduced RLNA as a modification of standard RLNS to prevent neural regrowth to the hemiparalyzed larynx and subsequent recurrence of SD. We have treated a total of 22 patients with RLNA, and now report a 3- to 7-year follow-up on 18 of these 22 patients. Resolution of symptoms was determined by routine follow-up assessment, perceptual voice analysis, and patient self-assessment. Sixteen of 18, or 89%, had no recurrence of spasms at 3 years after RLNA as determined at routine follow-up. Two of the 16 later developed spasms after medialization laryngoplasty for treatment of weak voice persistent after the avulsion. This yielded a total of 14 of 18, or 78%, who were unanimously judged by four speech pathologists to have no recurrence of SD at the longer follow-up period of 3 to 7 years. Two of these 4 patients were judged by all four analysts to have frequent, short spasms. The other 2 were judged by two of four analysts to have seldom, short spasms. Three of 18 patients presented with recurrent SD after previous RLNS. At the time of subsequent RLNA, each patient had evidence of neural regrowth at the distal nerve stump as demonstrated by intraoperative electromyography and histologic evaluation of the distal nerve stump. One remained free of SD following RLNA, 1 was free of spasms at 4 years after revision avulsion but developed spasms after medialization laryngoplasty, and the final patient developed spasms 3.75 years after revision RLNA. Medialization laryngoplasty with Silastic silicone rubber was performed in 6 of 18, with correction of postoperative breathiness in all 6, but with recurrence of spasm in 3. Spasms resolved in 1 of these with downsizing of the implant. We conclude that RLNA represents a useful treatment in the management of SD in patients not tolerant of botulinum toxin injections.

KEY WORDS — medialization laryngoplasty, recurrent laryngeal nerve avulsion, recurrent laryngeal nerve section, silicone rubber, spastic dysphonia.

INTRODUCTION

Since Dedo's[1] introduction of recurrent laryngeal nerve section (RLNS) in 1976 as the first effective treatment for adductor spastic dysphonia (SD), much progress has been made in the therapy for this troubling disorder. The long-term follow-up of 306 patients at 4 years found a recurrence of spasticity in 10% to 15% and overall patient satisfaction with the procedure in 90%, as measured by patient self-assessment.[2] Others found less favorable results in the long term. Aronson and DeSanto,[3] in a critical analysis of 33 patients, demonstrated an alarming failure rate, with the recurrence of spasms in 64% of these patients followed up for 3 years after RLNS. The recurrence of spasms was identified by perceptual analysis of audiotape recordings by a speech pathologist.[3] This study may have contributed to a significant reduction in the use of RLNS as a treatment for SD. The introduction of botulinum toxin (Botox) injections by Miller et al[4] and Blitzer et al[5] provided a welcome, effective nonsurgical alternative to RLNS. While Botox therapy has its disadvantages, including the necessity for repeated injections, its effectiveness in restoring normal voice for various periods of time with no destruction of function allowed it to supercede RLNS as the primary mode of treatment for SD.

Although Botox remains the therapy of choice for SD, not all patients are willing to undergo serial injections, for a variety of reasons. Many of our patients travel long distances, and return trips for additional injections are practically and economically unfeasible. The procedure itself is somewhat uncomfortable, and a few patients refuse repeat injections after experiencing the first. While virtually all patients achieve significant reduction in adductor spasms with a properly performed injection of botulinum toxin, the necessity for repeat injections to maintain this benefit causes some to seek alternative therapies. A definitive surgical procedure, if effective in the long term, would still be desirable for this

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TABLE 1. SELF-ASSESSMENT QUESTIONNAIRE

	Averaged Responses (n = 18)
Satisfaction	
1. I would recommend this surgery to others with SD.	6.8
2. I like my voice now better than before the operation.	7.2
6. I find it easy to communicate.	6.6
9. My voice sounds very much like a whisper when I am not trying to whisper.	1.6
10. OFTEN my voice sounds very much like a whisper when I am not trying to whisper.	1.4
14. I have to put forth great effort to communicate.	3.2
Voice stability	
5. My voice is more consistent on a day-to-day basis than before surgery.	7.3
11. Some days my voice is better than others.	5.8
17. The surgery helped my voice not change so much from day to day.	6.5
Recurrence of SD	
3. I have tightness in my voice that is similar to before surgery.	2.2
4. SKIP this question if you have NO tightness in your voice. This tightness is worse than before surgery.	0.4
7. I feel *strain* in my voice that is quite severe.	1.4
8. OFTEN I feel *strain* in my voice that is quite severe.	1.3
12. I feel *tremor* in my voice that is quite severe.	1.5
13. OFTEN I feel *tremor* in my voice that is quite severe.	1.5
15. I have voice *shutoffs* while I am talking that are quite severe.	1.6
16. OFTEN I have voice *shutoffs* while I am talking that are quite severe.	1.6

Responses were graded from 0 (strongly disagree) to 8 (strongly agree). Questions were presented to patients in random order as indicated by question number, but are grouped here in three categories of satisfaction, voice stability, and recurrence of spasm.

group of patients. Netterville et al[6] reported recurrent laryngeal nerve avulsion (RLNA) as a modification of RLNS in 1991. This procedure addressed regrowth of the recurrent laryngeal nerve (RLN) as one possible mechanism for recurrence of SD following RLNS. While not providing return of motion of the affected vocal fold, such neural regrowth restores muscular tone, providing a firm surface against which the mobile vocal fold could adduct and resulting in recurrence of adductor spasm. In theory, neural regeneration might be further impeded by completely avulsing each branch of the RLN from its muscular insertion, and by removing as much of the proximal RLN as possible. Histologic evidence of neural regeneration in 1 patient with recurrent SD after RLNS was presented, and short-term follow-up averaging 1.5 years was reported on 12 patients treated with RLNA without recurrence of spasm during this time period.[6] The following report extends this follow-up to a minimum of 3 years on 18 patients in order to match the follow-up of Aronson and DeSanto.[3] This will allow for a comparison of RLNS and RLNA in regard to the recurrence rate of SD. Postoperative voice assessment was accomplished by patient self-assessment, as well as by perceptual voice analysis by speech pathologists blinded to both clinical diagnosis and treatment paradigms. Further evidence of neural regeneration resulting in recurrence of SD following RLNS is presented in this report in 2 additional patients as confirmed by intraoperative electromyography (EMG) and histologic evaluation of the distal RLN stump. Our own record of treating over 400 patients with botulinum toxin while only 22 patients underwent RLNA attests to our firm opinion that RLNA should not replace botulinum toxin as the primary treatment for SD. The purpose of this report is twofold: first, to add further proof that neural regeneration is a significant cause of recurrent spasms after RLNS, and second, to demonstrate that RLNA is superior to RLNS in decreasing neural regrowth within a 3-year period. It is hoped that a less destructive procedure, such as partial myectomy as described by Genack et al,[7] may be shown to be more effective than neural removal in the treatment of this patient population who are unwilling to undergo serial injections of Botox.

MATERIALS AND METHODS

A detailed description of the operative procedure is found in the original report.[6] No additional modifications were made. In brief, the RLN was identified deep to the mobilized thyroid lobe via an approach similar to that used for RLNS or hemithyroidectomy. All branches of the RLN were then identified and traced to their muscular insertions deep to the cricopharyngeus muscle. Each branch was then carefully avulsed from this most distal point. The proximal RLN was then followed as it coursed deep to the

TABLE 2. PATIENT SUMMARY INFORMATION

Patient No.	*Age at Follow-up*	*Sex*	*Previous RLNS*	*Botox**	*Date of RLNA*	*Phonosurgery*	*Follow-up†*
1	50	F	None	×3	4/5/91	9/20/91	3 y 3 mo
2	74	F	None	None	4/5/89	None	5 y 3 mo
3	68	F	None	Multiple	6/18/90	None	4 y 1 mo
4	66	M	None	Multiple	4/1/91	None	3 y 3 mo
5	48	F	None	None	11/5/90	None	3 y 9 mo
6	53	F	None	×3	4/2/91	None	3 y 3 mo
7	38	F	None	×4	8/13/90	None	3 y 11 mo
8	46	M	None	None	4/7/87	None	7 y 3 mo
9	53	M	1985	×3	1/18/91	None	3 y 6 mo
10	64	M	None	None	6/9/87	10/7/87	7 y 1 mo
11	34	F	None	Multiple	11/5/90	12/11/92	3 y 8 mo
12	31	F	None	None	10/3/89	None	4 y 9 mo
13	40	F	None	×3	12/3/90	None	3 y 8 mo
14	43	F	None	Multiple	1/21/88	1/15/91	6 y 6 mo
15	57	F	1985	×4	10/1/90	None	3 y 9 mo
16	56	F	1986	None	4/6/88	7/17/92	6 y 3 mo
17	36	M	None	×3	10/2/90	3/91, 11/91	3 y 9 mo
18	47	F	None	×3	12/7/90	None	3 y 7 mo

RLNS — recurrent laryngeal nerve section, RLNA — recurrent laryngeal nerve avulsion.

*Number of Botox injections prior to RLNA.

†As determined at date of final voice recording.

clavicle and was sectioned in the upper mediastinum. The average length of nerve removed, including its distal branches, measured 9 cm. Medialization laryngoplasty with Silastic silicone rubber (Dow Corning) was performed as a secondary procedure in those patients bothered by excessive breathiness following RLNA. This technique was performed as reported by Netterville et al.[8] Patients were followed up postoperatively at 1 month, 3 months, 6 months, and 1 year. The 18-month postoperative evaluation included videostroboscopy, voice recordings, and a questionnaire. Follow-up at greater than 3 years consisted of a self-assessment questionnaire and recorded voice samples as described below.

Chart review identified 22 patients who underwent RLNA between April 7, 1987, and June 18, 1993. Eighteen are included with more than 3 years of follow-up. Details concerning previous treatments, date of RLNA, and any post-RLNA treatments were recorded. A self-assessment questionnaire was formulated and mailed to each patient (Table 1). Included in the mailing was a copy of the rainbow passage. All patients were then contacted by phone, and voice recordings were obtained, by means of a digital audiotape (DAT) recorder, of each patient reading the rainbow passage. Perceptual voice analysis was performed with the DAT recordings. The original DAT recordings of each patient's reading of the rainbow passage were assembled in random order on a single tape. This tape was then analyzed by four speech pathologists at the Vanderbilt Voice Center who were blinded to the identities of the patients. Voices were judged for abnormalities of pitch (modal level high or low, monotone, pitch breaks), loudness (modal level loud or soft, monotone, voice breaks), timing (rhythmic changes in pitch and loudness, interruptions, late voice onset, early voice offset), and quality (roughness, asthenia, breathiness, constriction, hoarseness, falsetto). Each category was rated on a four-point scale ranging from normal (0) to abnormalities noticeable only to professional examiners (1) and to those noticeable to nearly everyone (3). An overall assessment of presence or absence of spasm was made, and qualitative and quantitative descriptions of severity and frequency of spasms were given when spasm was identified. Results of patient self-assessment questionnaires and perceptual voice analysis were analyzed.

Assessment of neural regeneration after RLNS was initially tested in the office by the injection of 1% lidocaine hydrochloride into the tracheoesophageal groove at the site of the previous nerve section. At the time of subsequent RLNA an intraoperative EMG was performed after careful dissection to isolate the proximal and distal stumps of the RLN. Hook-wire electrodes were placed directly in the affected thyroarytenoid muscle transorally in 1 patient and via the cricothyroid membrane in another. Evoked potentials were recorded following bipolar stimulation of the identified distal RLN stump. Recordings were

TABLE 3. RESPONSE TO SELF-ASSESSMENT QUESTIONNAIRE

	Question Number																
Patient No.	*1*	*2*	*6*	*9*	*10*	*14*	*5*	*11*	*17*	*3*	*4*	*7*	*8*	*12*	*13*	*15*	*16*
1	8	5	6	2	2	7	6	8	6	4	3	4	4	4	4	3	1
2	4	8	4	0	0	0	8	8	8	0		0	0	0	0	0	0
3	8	6	8	3	1	0	8	8	8	4	1	2	2	1	1	0	0
4	8	8	8	0	0	0	8	8	8	6	0	0	0	0	0	0	0
5	8	8	8	0	0	0	8	0	8	0		0	0	0	0	0	0
6	8	8	8	0	0	0	8	4	8	0		0	0	0	0	0	0
7	8	8	8	2	2	0	8	5	8	0		0	0	0	0	0	0
8	8	8	8	0	0	0	8	5	8	0		0	0	0	0	0	3
9	8	8	8	1	1	1	8	7	7	1	0	0	0	0	0	0	0
10	8	8	5	0	0	1	8	7	5	2	1	1	1	0	0	0	0
11	7	7	6	1	1	2	5	6	7	4	2	4	3	4	4	4	3
12	***8***	***8***	***8***	***1***	***1***	***7***	***7***	***4***	***6***	***4***		***5***	***4***	***5***	***5***	***6***	***5***
13	8	4	6	2	2	2	8	1	8	0		0	0	0	0	0	0
14	***7***	***7***	***6***	***6***	***6***	***6***	***7***	***7***	***5***	***7***	***0***	***4***	***4***	***2***	***2***	***4***	***4***
15	***4***	***8***	***6***	***5***	***5***	***8***	***6***	***6***	***1***	***1***		***4***	***4***	***4***	***2***	***2***	***2***
16	***4***	***6***	***1***	***1***	***1***	***8***	***6***	***6***	***8***	***1***		***1***	***1***	***5***	***6***	***4***	***5***
17	4	7	6	2	2	5	7	6	2	5	1	1	1	1	1	6	6
18	4	8	8	2	2	4	8	8		0		0	0	1	0	0	0

Refer to Table 1 for text of questions and averaged response to each question. Responses are graded from 0 (strongly disagree) to 8 (strongly agree). Responses in bold italic type (patients 12, 14, 15, and 16) are from patients judged to have recurrent spasms by perceptual analysis. Refer to text of paper under Results for characterization of frequency and severity of spasms in each of these 4 patients.

made following stimulation of the RLN proximal to the site of previous nerve section, distal to the site of previous nerve section, and again proximal to the site of previous nerve section after repeat section of the RLN was performed prior to avulsion of the distal stump. Histochemical analysis of hematoxylin and eosin–stained preparations of the distal RLN stump was performed in both patients to assess the presence or absence of viable axonal tissue.

RESULTS

Twenty-two patients were treated with RLNA between April 1987 and June 1994; 18 are reported with follow-up ranging from 3 to 7 years. Data on these 18 are summarized in Table 2. Of these 18, 13 were female, and 5 male. Ages at the time of surgery ranged from 30 to 69 years, with ages at follow-up as listed in Table 2. Similar age distributions existed between males and females. All patients were previously treated, 12 with Botox, all with some form of speech therapy, and 3 with RLNS.

Self-assessment questionnaires were returned by all patients in the series. Averaged responses to each question are listed in Table 1, and individual responses are detailed in Table 3. Overall, responses were positive, with 13 strongly recommending the procedure to others, while 5 were neutral in their recommendation. Postoperative voices were felt to be more consistent on a day-to-day basis. Three patients agreed they had a tightness in their voices similar to that before surgery, 4 gave a neutral response to this question, and the remaining 11 denied any residual tightness. Two patients reported frequent voice shutoffs. Six reported great effort was still required to communicate, although it was not specified whether this was secondary to recurrent spasms or to breathy voice.

Perceptual analysis of postoperative voices in random order by blinded speech pathologists revealed that all patients had some degree of abnormality as judged by at least two of four analysts. General agreement between examiners was found for both the type and severity of identified abnormalities. This relative uniformity of responses between independent skilled observers favored the reliability of each analyst's judgments. Ten patients were thought to have pitch abnormalities: 3 with severe, 4 with moderate, and 3 with mild pitch abnormalities. Nine patients were thought to have loudness abnormalities: 1 with severe, 4 with moderate, and 4 with mild abnormalities. Three patients were thought to have mild to moderate timing abnormalities. Seventeen patients were thought to have quality abnormalities: 8 with severe, 5 with moderate, and 4 with mild quality abnormalities. Fourteen of 18 patients (78%) were unanimously judged by all four analysts to be without recurrence of SD. Two patients (Nos. 15 and 16) were judged by four of four analysts to have frequent, short spasms, while 2 patients (Nos. 12 and 14) were thought to have seldom, short spasms by

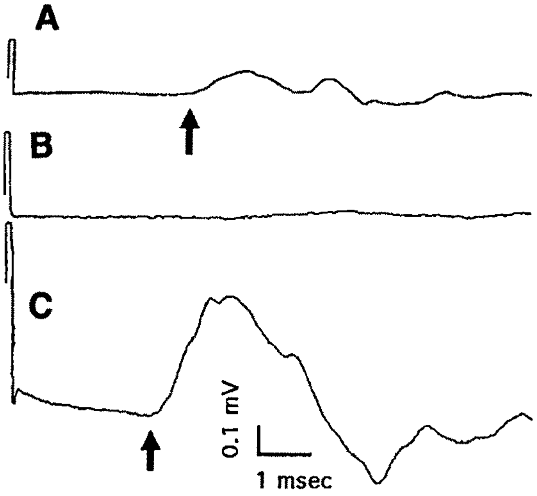

Fig 1. Evoked electromyography potentials recorded from thyroarytenoid muscle in patient 9 following stimulation of recurrent laryngeal nerve (RLN) proximal to neuroma (A), proximal to site of neuroma after resection of neuroma (B), and distal to site of neuroma after resection (C). Potential evoked by proximal nerve stimulation (arrow, A) immediately disappeared after resection of neuroma (B), indicating that nerve fibers in RLN had regenerated through neuroma to reinnervate laryngeal muscles. Potential evoked by distal nerve stimulation (arrow, C) was shorter in latency and larger in amplitude than that produced with proximal stimulation. Shorter latency could be explained by shorter distance compound action potential would have to travel before muscle activation. Greater amplitude suggested that some regenerated nerve fibers from RLN could only conduct action potentials with stimulation distal to neuroma. Apparently, when these fibers were activated more proximally, their action potentials were blocked by neuroma, indicative of neurapraxia. Alternative explanation was that axons from source other than RLN had regenerated and effectively invaded distal stump. However, stimulation of ansa hypoglossi branches or tissue surrounding neuroma was ineffective in producing evoked EMG response, supporting first explanation, that RLN was solitary source of muscle reinnervation.

two of four analysts. However, 2 of these 4 patients (Nos. 14 and 16) were judged at routine follow-up assessment to be free of spasms at 3 and 4 years, respectively, after their RLNA. Because of weakness of their voices, they underwent medialization laryngoplasty, with spasms returning later as described below. Thus, at 3 years post-RLNA, 16 of 18 (89%) were free of spasms. These 4 patients are highlighted in Table 3. Correlation of patient self-assessment with perceptual analysis was greatest with regard to effort to communicate. All 4 patients judged to have recurrent adductor spasms reported that significant effort was still required to communicate. Only 2 of the remaining 14 patients not judged to have recurrent spasms agreed that great effort was still required to communicate.

Six of 18, or 33%, underwent medialization laryngoplasty with Silastic silicone rubber for persistent breathiness following RLNA. Four of these 6 (Nos. 1, 10, 11, and 17) were judged by all speech pathologists to be without recurrence. One of these 4 (No. 17) did have mild recurrence of spasm following Silastic medialization performed 5 months after RLNA. A single Botox injection was tried without success. Subsequent revision Silastic medialization to reduce the size of the Silastic implant resulted in resolution of spasms. The remaining 2 patients in the medialization group (Nos. 14 and 16) were judged by two of four, and four of four analysts to have recurrent SD. In patient 14, medialization laryngoplasty with Silastic silicone rubber was performed 3 years following RLNA for persistent breathiness. While routine follow-up assessment judged this patient to be free of spasm prior to laryngoplasty, spasms recurred within 1 year of medialization, and persisted at the 6.5-year follow-up. This patient, who is a public speaker, stated that the spasms were only bothersome for 1 week around the time of her menstrual period. A revision laryngoplasty was suggested with placement of a smaller implant; however, the patient did not feel her voice was bothersome enough to warrant revision surgery. Patient 16, who was described in our original report, developed severe recurrence of spasms 1 year after previous RLN section. We revised her original procedure with an RLNA, with resolution of her spasms and no recurrence 4 years later. Because of difficulty in communication secondary to persistent breathiness, she underwent medialization laryngoplasty with Silastic silicone rubber 4 years after RLNA. This patient was judged on routine follow-up assessment to be free of spasm at the time of medialization, but developed recurrent spasms within 5 months of the laryngoplasty. She has received four supplemental Botox injections in 2.5 years of follow-up after medialization. We suggested revision of the implant size. Because of insurance limitations from her managed care provider, she underwent a revision at another institution, with no improvement in her spasms. She continues receiving Botox with moderate success.

Two other patients underwent RLNA after previous RLNS with recurrent SD. The RLNS was performed 5 and 6 years prior to RLNA in these 2 patients (Nos. 15 and 9). Both reported initial relief of spasm following nerve section, but subsequent recurrence of spasms developed in both by 3 years. Botox was tried in both, but spasms persisted. Intraoperative EMG was performed prior to neural avulsion as described above. In both patients the RLN was identified and noted to be grossly intact, with evidence of neuroma formation at the apparent site of previous nerve section. The RLN was stimulated proximal to the neuroma, distal to the neuroma after resection of the neuroma, and again proximal to the neuroma after

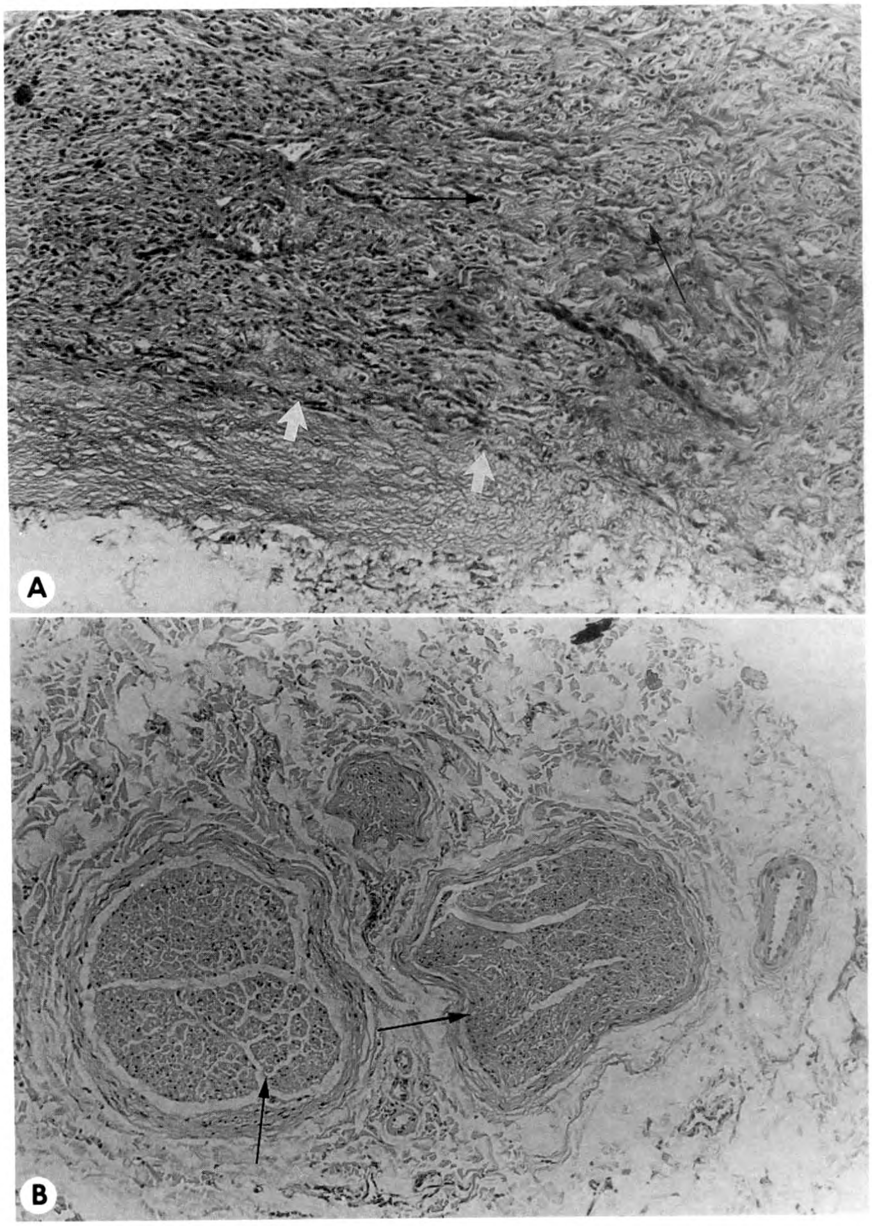

Fig 2. Sections of distal recurrent laryngeal nerve stump (H & E, original ×10). A) Patient 9. B) Patient 15. Thin black arrows indicate regenerated axon profiles within nerve distal to neuroma identified at time of surgery in each patient. In longitudinal section (A), poorly delineated border of funiculus is indicated by broad white arrows. At this level, some axon profiles are cut at oblique angle. Well-organized bundles of regenerated axons representing individual funiculi can be better appreciated in cross section of nerve, as shown in B.

resection of the neuroma but prior to avulsion of the distal stump. Figure 1 illustrates EMG findings from 1 of these 2 patients (No. 9). Histologic analysis of the RLN stump confirmed the presence of regenerated axons within the distal nerve in both patients (Fig 2). Of these 2 patients, 1 (No. 9) was judged by all four speech pathologists to be free of recurrence, while the other (No. 15) was felt by all four analysts to have recurrent spasms. Medialization laryngoplasty was not performed in either of these patients.

DISCUSSION

Therapy for SD underwent a significant advance with the introduction of RLNS in 1976.[1] The previously ineffective attempts at both speech therapy and psychotherapy for what was felt to be a functional disorder were called into question by this dramatic approach to surgically alter a physiologic abnormality of laryngeal function. Speech therapy then assumed its role as a necessary and effective therapeutic modality for SD after partial interruption of laryngeal hyperfunction had been accomplished. The initial effectiveness of RLNS prompted the search for other ways to alter this laryngeal hyperfunction. The application of Botox to the treatment of SD represented a direct consequence of the early success of RLNS, substituting a pharmacologic interruption of muscular function for a surgical one.

That RLNS has fallen out of favor is a consequence of long-term follow-up of the procedure in a number of series suggesting significant recurrence rates of SD at 3 years and beyond.[3] This literature is reviewed in our original report.[6] Successful application of Botox as effective therapy for SD, coincident with the reports of late recurrence of SD following RLNS, established Botox as the therapy of choice.[9] The late failure of RLNS was puzzling, given that the affected vocal fold remained clinically immobile in these patients. This was felt by most authors to be secondary to a progressive increase in function of the contralateral vocal fold.[3] While such increased function may well play a role, the alternative explanation of partial reinnervation of the denervated hemilarynx correlates well with the time course of recurrence, is well demonstrated in experimental animals, and is testable in those patients with recurrent SD after RLNS. The work of Crumley and McCabe[10] clearly showed regeneration with reconstitution of the distal nerve in sectioned and suture-ligated RLNs in dogs. Early reports of reexploration for revision RLNS following return of SD suggested the possibility of a reconstituted RLN, and noted resolution in symptoms following repeat resection.[11,12] Most convincing is a report by Ludlow et al[13] of 5 patients with recurrent SD following RLNS in whom EMG demonstrated equal levels of thyroarytenoid muscle activation on the operated and nonoperated sides despite an absence of physiologic motion of the vocal fold on the operated side. All 5 of these patients achieved significant reduction in speech symptoms following injection of Botox on the operated side, with 2 patients also receiving injections on the nonoperated side.[13] One patient was presented in the original description of RLNA with histologic evidence of neural regeneration in the distal RLN stump following previous RLNS.[6] Support for reinnervation as a cause of recurrent SD following nerve section is provided in the current report by data from 2 additional patients. Both underwent RLNA for recurrent SD after previous RLNS. Both demonstrated gross evidence of neural regeneration with neuroma formation at the site of previous nerve section joining proximal and distal RLN stumps. Histologic evidence of axonal regeneration within the distal nerve stump supported this clinical observation (Fig 2). Electrophysiologic evidence of reinnervation was obtained in both patients with intraoperative EMG recordings of evoked motor unit potentials from the thyroarytenoid muscles on the operated sides. That these motor unit potentials were recorded with longer latencies and broad waveforms was consistent with stimulation of axons that regenerated through neuroma. Disappearance of evoked potentials with stimulation of the proximal RLN following resection of the region of the neuroma provided further confirmation of reinnervation via the proximal RLN in both of these cases (Fig 1). No detectable motion of the ipsilateral vocal fold was seen in either patient prior to RLNA. Although regeneration did not provide effective return of vocal fold motion, it likely resulted in sufficient return of vocal fold tone to reinstate adductor spasms. This can occur secondary to significant neural regrowth with increased motor tone within the thyroarytenoid during phonation. Even in the absence of motion, if the paralyzed vocal fold is resting near the midline, this motor tone is sufficient to create a firm surface against which the mobile vocal fold adducts, resulting in vocal spasms.

We suspect that even those with RLNA develop some reinnervation; however, it is not usually sufficient to result in recurrent spasm. This point was demonstrated in patient 11, who developed a very weak, breathy voice after her RLNA. Two years after her nerve avulsion, she underwent medialization laryngoplasty. During the procedure, weak voluntary motor unit potentials were observed within the paralyzed thyroarytenoid muscle by the use of hook-wire electrodes. She did not demonstrate adductor spasms before or after the laryngoplasty, which was performed in December 1992. A slightly undersized implant was used in this patient to decrease the chance of recurrent spasms.

As a modification of RLNS, RLNA was developed to further hinder this process of reinnervation. While other authors removed fairly short segments of RLN (2 cm on average), with RLNA the entire course of the RLN accessible in the neck is removed (averaging 9 cm). This modification is logical, but is not the critical aspect of RLNA. Reinnervation of the distal RLN stump may be derived from a number of sources, not merely the proximal RLN. Certainly, if the proximal RLN remains in close proximity to the distal stump, then reinnervation may occur more readily, as was seen in both cases of recurrent SD following RLNS described above. Removal of a long segment of RLN into the upper mediastinum should effectively prevent this. Reinnervation, however, may also occur from other motor nerves in the area, notably branches of the ansa cervicalis or ansa hypoglossi injured during the original dissection. Axons from these sources may easily regenerate in a misdirected fashion, seeking the distal RLN stump. Neural avulsion seeks to answer this problem. Traction on each distal nerve branch of the RLN stump until it is pulled out of the laryngeal muscles removes all normal neural pathways into the hemilarynx.[6]

Dedo[14] attributes his low recurrence rate to suture ligation of the proximal and distal cut ends of the RLN. Crumley and McCabe's[10] work indicates that in the experimental animal, suture ligation of the RLN does not prevent regeneration of the nerve. The technique of RLNA circumvents this dilemma by surgically removing the distal RLN stump. It accomplishes denervation of the hemilarynx, and is designed to disrupt the physiologic process of reinnervation by widely separating the proximal motor nerve from its target organ, and by removing all residual motor nerve fibers from the denervated target organ that might otherwise accept regenerating axons from other sources. Theoretically, this should further impede reinnervation of the hemilarynx. Although there are other routes by which the hemilarynx may reinnervate, the low recurrence rate of spasms in this study group implies they are not as effective as direct regrowth via the distal RLN stump.

That an objective method of analyzing the SD voice is not universally agreed upon is evidenced by the variety of techniques reported. Patient self-assessment is an important determinant of the success of any procedure — some would say the most important factor. However, it is entirely subjective, and while important, must be viewed with caution when used alone as a tool for objective comparisons between techniques and groups of patients. Perceptual analysis by trained speech pathologists represents a more objective technique, but one that remains difficult to standardize. While acoustic analysis holds promise as the most objective measure, and one that could easily be applied to a wide variety of patient populations treated with a number of different modalities, agreement on which parameters provide accurate clinical correlates is lacking. Eskenazi et al, in an attempt to relate six perceptual parameters with five acoustic parameters, found that "acoustic measures used as predictors of quality appear to represent only a fraction of the set of all measures used by the human listener."[15(p304)] Zwirner et al[16] found in SD patients treated with Botox a correlation of only two acoustic parameters with perceptual analysis: the standard deviation of fundamental frequency and the voice break factor. In the current study we combined subjective patient self-assessment with perceptual analysis by trained speech pathologists to assess our results. This method is comparable with those of previous reports on RLNS.

With regard to patient self-assessment, 13 of 18, or 72%, strongly recommended the procedure to others, with the remaining 5 being more neutral in their recommendation. As illustrated in Table 1 and Table 3, the overall responses at 3 to 7 years post-RLNA were quite positive. The post-RLNA voice was felt to be more consistent, with only 2 patients reporting frequent voice shutoffs. This result was further supported by follow-up clinical assessment, as well as perceptual analysis by blinded speech pathologists. Clinical assessment judged 16 of 18 (89%) free of spasms at 3 years post-RLNA. Two of these 16 patients subsequently developed mild spasms after medialization laryngoplasty, decreasing more long-term results to 14 of 18 (78%) free of spasms. These 14 were unanimously judged by four speech pathologists to be free of spasms at 3 to 7 years. Correlation of perceptual analysis with self-assessment was greatest for effort to communicate, with all 4 identified to have recurrent SD by perceptual analysis reporting persistent significant effort to communicate. Only 2 of the remaining 14 patients judged free of SD responded in the affirmative that effort to communicate was still great.

The postoperative breathiness that can occur following RLNS or RLNA is significant. Six of 18 patients underwent a vocal fold medialization procedure following RLNA in this series. The procedure improved breathiness in all 6, but contributed to recurrence of spasm in 3. In 1 of these 3, a revision procedure to reduce the size of the Silastic block was performed, with subsequent elimination of spasms. The remaining 2 patients had spasm-free intervals following RLNA of 3 and 4 years, but were troubled by breathiness. Recurrent spasm developed within 1 year of subsequent Silastic medialization in both patients. Medialization laryngoplasty with Silastic silicone rubber corrects the breathiness that sometimes occurs following RLNA, but may result in recurrence of spasms. The mechanism of how vocal fold medialization might exacerbate quiescent SD is not certain. It is obvious, however, that recreating a firm surface for the normally mobile vocal fold to adduct against may result in the recurrence of spasms. It was evident during the medialization procedures that pushing in too far within the window resulted in recurrent spasms. Thus, each patient was undercorrected. Even with this undercorrection, 3 patients developed recurrent spasms within 6 months. The reason for this delayed recurrence after medialization is unclear; however, it is reversible by further downsizing the implant, as demonstrated by patient 17. The resolution of breathiness without recurrence of spasm in 3 others demonstrated that a successful balance could be achieved between sufficient medialization to produce strong voice and overmedialization that would lead to spasm. In this small series, clinical predictors of failure following laryngoplasty were not identified. It would appear, from observing both groups with recurrence of SD — those with recurrence after presumed neural regrowth and those with recurrence after medialization laryngoplasty — that the major factor leading to spasms is the firmness of a paralyzed vocal fold positioned close enough to the midline so that the mobile vocal fold can adduct firmly against it. When the reinnervation is not sufficient to position the paralyzed vocal fold near the midline, too large an implant during laryngoplasty can compress the residual muscle to the midline, resulting in recurrence of spasms. Explaining recurrent spasm by overmedialization alone, however, likely represents an oversimplification, within the complex physiologic milieu of the partially denervated hyperfunctional larynx in which laryngeal framework surgery is attempted.

Long-term follow-up in 3 patients treated with RLNA following previous RLNS revealed 1 with no recurrence of spasms 4 years after avulsion. The second developed recurrent spasms 4.5 years after RLNA, probably partially because of medialization laryngoplasty performed 4 years after the avulsion for a weak and breathy voice. This patient's long spasm-free interval (4 years) prior to laryngoplasty suggested the medialization procedure as a likely cause of recurrent spasms as described above. The final patient developed recurrent spasms 3.75 years after RLNA. In this case no cause of recurrence has been identified. This patient (No. 15) can be grouped with 1 other (No. 12) as the 2 patients judged to have recurrent SD following RLNA in whom no laryngeal framework surgery was performed postoperatively. We have not had the opportunity to perform EMG on either patient. In both of these patients, however, the development of renewed adductor spasms logically should be attributed to reinnervation of the paralyzed hemilarynx despite the impediments to reinnervation created by the avulsion procedure. This yields a failure rate of 11% for RLNA at 3 to 7 years as defined by patients with recurrent spasm in whom no other suspected cause of recurrent spasm was identified.

Although the ability of the patients to communicate was significantly improved, all patients had some form of voice abnormality. No patient had a normal voice after RLNA. All patients retained abnormalities of pitch, loudness, timing, and quality following treatment. This is a very important factor to discuss preoperatively with patients who are considering laryngeal deinnervation for the treatment of SD. With botulinum toxin treatments, in spite of the poor, breathy phase soon after the injection and the phase of recurrent spasms before the next injection, there is a significant period of normal voice in most patients. Because the loss of normal voice is universal and the effect of RLNA is permanent, we convince most patients considering nerve avulsion to continue with botulinum toxin therapy. There are very few patients who are not served well with Botox. We work with each patient to find a regimen of injection, whether unilateral, alternating unilateral, or bilateral, that will result in normal voice with the shortest abnormal voice period during the injection cycle. Nerve avulsion should be reserved for the select patient who does not benefit from or tolerate Botox and who is very well informed about the issues discussed in this paper. An exception is the patient who has developed recurrent spasms after a previous nerve section. We highly recommend revision surgery with RLNA in patients whose vocal cord is already paralyzed. With this limited criterion the ability to improve communication with RLNA is relatively dependable. The major downside of the procedure is a high percentage of patients with a weak, breathy voice that must be addressed to improve their communication further.

CONCLUSION

Long-term follow-up of RLNA revealed that 72% to 78% of patients retained clear benefit from the procedure beyond 3 years as measured by patient self-assessment and perceptual analysis by blinded speech pathologists. Evidence of laryngeal reinnervation as a cause of recurrent SD following RLNS is presented. Postoperative breathiness, occurring in 6 of 18 patients, was improved in all patients by subsequent medialization of the vocal fold. Medialization, however, may have contributed to recurrence of spasm in 3 of these 6. While not supplanting Botox injections as the preferred therapy for SD, RLNA yields a more consistent long-term elimination of adductor spasms than other surgical therapies, and represents a useful procedure for those patients not tolerant of Botox injections.

REFERENCES

1. Dedo HH. Recurrent laryngeal nerve section for spastic dysphonia. Ann Otol Rhinol Laryngol 1976;85:451-9.
2. Dedo HH, Izdebski K. Intermediate results of 306 recurrent laryngeal nerve sections for spastic dysphonia. Laryngoscope 1983;93:9-15.
3. Aronson AE, DeSanto LW. Adductor spastic dysphonia: three years after recurrent laryngeal nerve resection. Laryngoscope 1983;93:1-8.
4. Miller RH, Woodson GE, Jankovic J. Botulinum toxin injection of the vocal fold for spasmodic dysphonia: a preliminary report. Arch Otolaryngol Head Neck Surg 1987;113:603-5.
5. Blitzer A, Brin MF, Fahn S, Lovelace RE. Localized injections of botulinum toxin for the treatment of focal laryngeal dystonia (spastic dysphonia). Laryngoscope 1988;98:193-7.
6. Netterville JL, Stone RE, Rainey C, Zealear DL, Ossoff RH. Recurrent laryngeal nerve avulsion for treatment of spastic dysphonia. Ann Otol Rhinol Laryngol 1991;100:10-4.
7. Genack SH, Woo P, Colton RH, Goyette D. Partial thyroarytenoid myectomy: an animal study investigating a proposed new treatment for adductor spasmodic dysphonia. Otolaryngol

Head Neck Surg 1993;108:256-64.

8. Netterville JL, Stone RE, Luken ES, Civantos FJ, Ossoff RH. Silastic medialization and arytenoid adduction: the Vanderbilt experience. A review of 116 phonosurgical procedures. Ann Otol Rhinol Laryngol 1993;102:413-24.

9. Miller RH, Woodson GE. Treatment options in spasmodic dysphonia. Otolaryngol Clin North Am 1991;24:1227-37.

10. Crumley RL, McCabe BF. Regeneration of the recurrent laryngeal nerve. Otolaryngol Head Neck Surg 1982;90:442-7.

11. Wilson FB, Oldring DJ, Mueller K. Recurrent laryngeal nerve dissection: a case report involving return of spastic dysphonia after initial surgery. J Speech Hear Disord 1980;45:112-8.

12. Schiratzki H, Fritzell B. Treatment of spasmodic dysphonia by means of resection of the recurrent laryngeal nerve. Acta Otolaryngol [Suppl] (Stockh) 1988(suppl 449):115-7.

13. Ludlow CL, Naunton RF, Fujita M, Sedory SE. Spasmodic dysphonia: botulinum toxin injection after recurrent nerve surgery. Otolaryngol Head Neck Surg 1990;102:122-31.

14. Dedo HH. Surgery of the larynx and trachea. Toronto, Canada: BC Decker, 1990:29-59.

15. Eskenazi L, Childers DG, Hicks DM. Acoustic correlates of vocal quality. J Speech Hear Res 1990;33:298-306.

16. Zwirner P, Murry T, Woodson GE. Perceptual-acoustic relationships in spasmodic dysphonia. J Voice 1993;7:165-71.

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AUTOLOGOUS FAT INJECTION FOR UNILATERAL VOCAL FOLD PARALYSIS

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This study evaluates the efficacy of autologous fat injection for medialization of the paralyzed vocal fold. In 21 patients with unilateral vocal fold paralysis, autologous abdominal fat was injected into the thyroarytenoid muscle to achieve medialization. All patients were followed up with serial videolaryngoscopy and voice evaluation. At 2 months' follow-up, the voice was judged to be excellent in 10 patients, slightly breathy but significantly better than the preoperative voice in 6 patients, and markedly breathy in 4 patients. At 3 to 4 months' follow-up, of the 10 patients with excellent results, 5 maintained an excellent voice, 3 had developed slight breathiness, and 1 had developed severe breathiness. Long-term (6 to 12 months) results were available in 11 patients, and all of them maintained the same voice quality that was noted during the 3 to 4 months' examination. Magnetic resonance imaging of the larynx was obtained in 7 patients at intervals ranging from 1 to 7 months and compared to the baseline scan obtained at 1 week postoperative to assess the amount of fat remaining in the muscle. The images showed fat volume to persist, but a decrease in the fat signal was observed over time. The results suggest that the duration of medialization with autologous fat is variable, but appears to last at least 2 to 3 months. This loss of volume after 3 months seems to be due to absorption of the fat and possibly muscle atrophy. Autologous fat injection is relatively safe and easy to perform, and is an ideal method of temporary vocal fold medialization in patients in whom return of vocal fold function is expected.

KEY WORDS — autologous fat injection, medialization procedure, vocal fold paralysis.

INTRODUCTION

Incomplete glottic closure due to unilateral vocal fold paralysis can result in aspiration, inability to produce an effective cough, and breathy hypophonia. Such problems can be disabling, or potentially life-threatening, and significantly affect the quality of life. A variety of techniques have been described for improving glottic incompetence resulting from unilateral vocal fold paralysis: 1) injection of various substances, most commonly Teflon or Gelfoam, 2) laryngeal framework surgery, such as medialization laryngoplasty (thyroplasty) or arytenoid adduction, and 3) laryngeal reinnervation procedures. Although effective, all of these methods have limitations and potential complications. Vocal fold injection is a relatively simple and quick procedure; however, only a few substances are available for injection and each has its own limitations. Teflon has been shown to be associated with granuloma formation and scarring, which can result in poor voice quality.[1,2] More recently, it has been on and off the market and possibly may no longer be manufactured in the future. Gelfoam paste has been used for temporary vocal fold paralysis and has been reported to last only an average of 6 to 8 weeks.[3] Currently, Gelfoam is not available as a paste; therefore, a mixture of the powder form and saline is used for injection, which appears to last only for approximately 1 month. Collagen has been shown to be effective for vocal fold medialization; however, some loss of volume does occur over time with this substance.[4] Furthermore, the use of collagen requires preoperative skin testing and is contraindicated in individuals demonstrating a hypersensitivity reaction. Medialization laryngoplasty requires the use of an alloplastic implant and may be associated with extrusion of the implant.[5] Arytenoid adduction is technically difficult to perform, and complications such as pharyngocutaneous fistula and airway obstruction have been reported.[5] Reinnervation of a paralyzed unilateral vocal fold by anastomosis of the ansa hypoglossi to the recurrent laryngeal nerve was popularized by Crumley[6] and probably yields the best voice quality because the reinnervation maintains muscle bulk, restores tension to the vocal ligament, results in forces of pull on the arytenoid cartilage that maintain the vocal ligament in an adducted position, and has the potential to restore movement of the paralyzed vocal fold.[6] This technique, however, has disadvantages and limited applications. The distal stump of the recurrent laryngeal nerve may not be available for anastomosis following thyroidectomy, as the nerve may have been resected or be encased in

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CAUSES OF VOCAL FOLD PARALYSIS

Penetrating neck trauma	
Gunshot wound	6
Stab wound	4
Excision of skull base tumor	4
Intracranial surgery	2
Lung transplant	2
Carotid endarterectomy	1
Intubation	1
Idiopathic	1

extensive scar tissue. Furthermore, it is a longer procedure, and a wait of at least a few months is required to attain the desired results.

Ideally, vocal fold medialization should be accomplished with a simple medialization technique with a low risk of associated complications. Injection of an autologous substance with a low absorption rate would seem to fit these criteria. A few clinical cases of autologous fat injection for unilateral vocal fold paralysis have previously been reported.[7,8] The results in these reports indicate that the improvement in the voice quality is permanent in many of the patients. As autologous fat injected into various sites in the body has been clearly shown to resorb with time, it is unclear as to whether maintenance of the improved voice quality is truly due to the injected fat or compensation with time in these patients. This study evaluates the results of autologous fat injection for unilateral vocal fold paralysis in 21 patients by means of voice evaluation, videostroboscopy, and serial magnetic resonance imaging (MRI).

MATERIALS AND METHODS

Twenty-one patients underwent injection of autologous fat for unilateral vocal fold paralysis at the University of Southern California affiliated hospitals (LAC-USC Medical Center and USC University Hospital). The causes of the vocal fold paralysis are shown in the Table. The indications for surgery in these patients were 1) disabling breathy hypophonia, 2) aspiration, and 3) inability to produce an effective cough following lung transplantation. All of the patients had preoperative videolaryngoscopy with a flexible or a 90° rigid fiberoptic scope, which documented the paralyzed vocal fold and a glottic gap.

Operative Technique. The fat was obtained from the lower abdomen, just below the umbilicus. In 18 of the patients, it was harvested through a 2-cm incision and minced into 1- to 3-mm pieces with a No. 10 blade. All surrounding fascia and connective tissue were then meticulously removed, leaving only fat globules. In the other 3 patients, the fat was obtained by liposuction with a 4-mm-diameter cannula attached to a 30-mL syringe. The fat was placed on fine mesh gauze and thoroughly irrigated with lactated Ringer's solution in order to rinse away remaining blood elements. The fat globules were then loaded into a Bruning syringe with an 18-gauge needle. The injection was performed under general anesthesia with either apneic technique or jet ventilation. Two sites of injection were used, the first site being just lateral to the vocal process and the second site at the mid-fold. The needle was always directed such that the fat would be injected into the thyroarytenoid muscle. Every attempt was made to overinject so that the injected fold was medialized past the midline, as some resorption of the fat was expected.

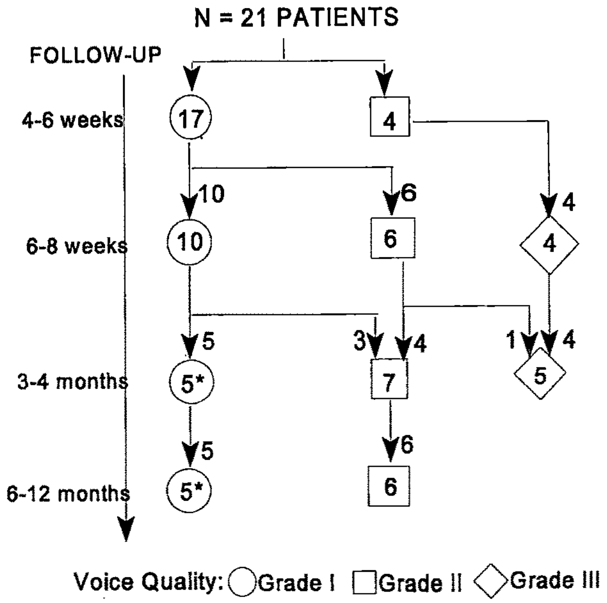

Fig 1. Summary of clinical course of 21 patients injected with autologous fat for unilateral vocal fold paralysis. * — One patient had return of vocal fold function at 3 months, and another had partial return of function at 6 months.

Surgery was performed as an outpatient procedure in all cases. The patients were followed up at 1 week postoperatively, then at intervals ranging from 4 to 6 weeks for 6 months, and then every 3 months thereafter for 1 year. The quality of the voice was assessed at the time of follow-up by asking the patient to rate his or her voice quality, specifically with respect to vocal fatigue and the ability to project the voice. Videostroboscopy was performed during each follow-up visit. In 7 patients, MRI of the larynx was obtained to look for evidence of remaining injected fat. A baseline scan was obtained 1 week after injection and repeated at 1 month and again between 3 and 7 months. T1-weighted images were obtained, as they give the greatest signal intensity for fat.

RESULTS

No complication occurred related to the injection procedure, such as hematoma, inflammatory response,

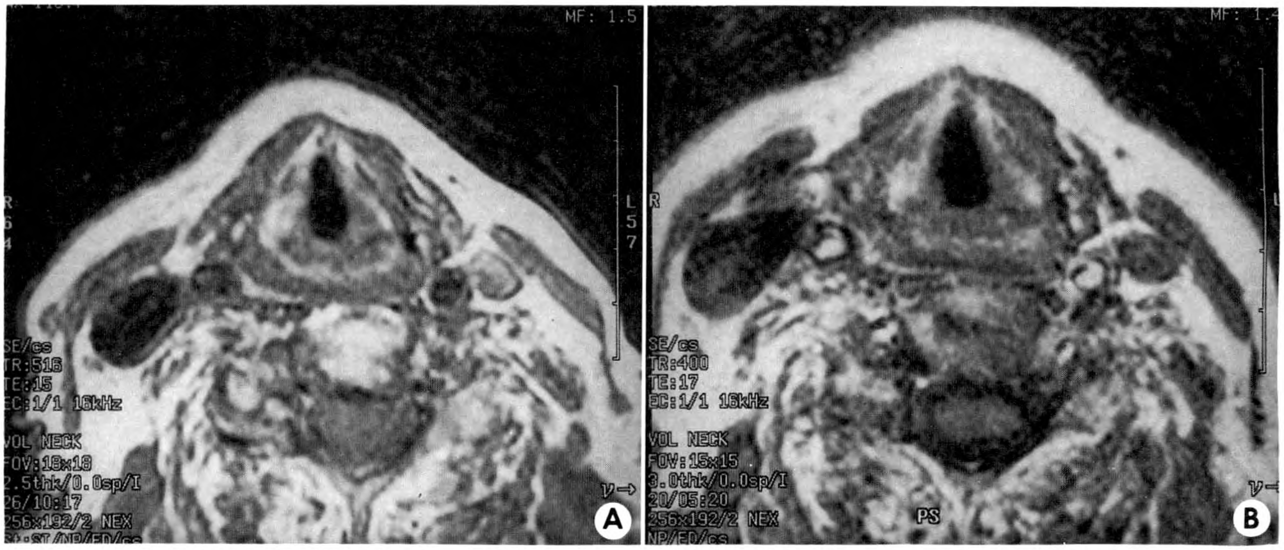

Fig 2. Magnetic resonance images of larynx. Note bright signal intensity in right vocal fold, which is consistent with presence of fat. A) Scan performed at 6 weeks after injection, which was identical in appearance to that performed at 1 week postoperative. B) Scan performed at 5 months postoperative, showing persistence, but slight loss of volume, of injected fat.

airway obstruction, or aspiration of fat into the tracheobronchial tree. None of the patients sustained donor site complications. For data analysis, the quality of the postoperative voice was graded as follows: grade I, excellent voice without breathiness or vocal fatigue; grade II, slightly breathy voice without vocal fatigue, but significantly better than the preoperative voice; and grade III, markedly breathy voice. The results are summarized in Fig 1. At the 4- to 6-week interval, 17 of the patients had grade I voice and 4 had grade II voice. Two of these 4 patients with grade II voice had resection of their vagus nerve for neoplasm, 1 had lung cancer, and the other had cautery injury to the recurrent laryngeal nerve during lung transplantation. At 6 to 8 weeks, of the 17 patients with grade I voice during the prior evaluation, 10 maintained the same voice quality, 6 developed mildly breathy voice (grade II), and 1 was lost to follow-up. All 4 patients who had grade II voice during the previous evaluation developed grade III voice and subsequently underwent thyroplasty and/or arytenoid adduction. At 3 to 4 months, in the 10 patients who previously had grade I voice, 5 remained unchanged, 3 developed grade II voice, and 2 were lost to follow-up. One of the 6 patients who had grade II voice during the prior evaluation was lost to follow-up, 4 maintained the same voice quality, and 1 developed breathiness (grade III). The patient who developed grade III voice also underwent thyroplasty subsequently. Thus, of the 13 patients who were evaluated at 3 to 4 months, 5 had grade I voice, 7 had grade II, and 1 had grade III. Eleven patients were evaluated between 6 and 12 months, and their voice remained unchanged compared to the 3- to 4-month evaluations: 5 maintained grade I voice and 6 maintained grade II voice. In summary, in 17 patients who were available for follow-up at 3 to 4 months, the voice was judged to be excellent to satisfactory (grades I and II) in 12 patients, and breathy in 5. The voice remained stable thereafter in those patients who were available for follow-up at 6, 9, and 12 months.

Videolaryngoscopy showed complete return of vocal fold function in 1 patient at 3 months and partial return of function in another at 6 months. Both of these patients maintained a grade I voice for the duration of the paralysis, and the voice remained normal following return of vocal fold function. All of the other patients who had grade I voice beyond 6 months had persistent paralysis with good approximation of the vocal folds, a symmetric mucosal wave, and no compensatory supraglottic hyperadduction. In the 7 patients who ultimately developed grade II voice at 3 to 4 months, 2 had good approximation of the anterior fold but a moderate-size posterior gap, 1 had shortening of the paralyzed vocal fold with the arytenoid pulled anteriorly, and the other 4 had small midglottic gaps. All of the patients who developed grade III voice were observed to have progressively larger glottic gaps with time.

Laryngeal MRI scans were obtained in 7 patients: 1 patient who maintained a grade I voice beyond 6 months and 6 patients who developed a grade II voice. In all 7 patients, the MRIs that were obtained at the 4- to 6-week interval showed persistence of high signal intensity in the thyroarytenoid muscle, consistent with the presence of fat (Fig 2A). These scans were similar to the baseline scans that were obtained at 1 week postoperative. The fat signal intensity was also present in scans that were obtained between 3 and 6 months; however, some reduction in the volume was observed (Fig 2B).

DISCUSSION

The concept of using autologous fat for medialization of a paralyzed vocal fold in humans was first introduced by Mikaelian et al[7] in 1991. They reported good results in 3 patients, with follow-up ranging from 3 to 12 months. Brandenburg et al[8] in 1992 reported on their experience with this technique in 11 patients, with follow-up ranging from 5 to 42 months. The injected vocal fold remained medialized in 10 patients, and 1 patient required reinjection. In contrast to these reports, a large percentage of our patients did not experience such good long-term results. Maintenance of the bulk and medialization would not be expected to be permanent, as previous studies on the long-term fate of injected fat in various head and neck sites other than the larynx indicate that 20% to 90% of injected fat will ultimately be resorbed.[9] The results of the voice quality and videolaryngoscopic findings from our study indicate that the duration of medialization is quite variable. Seventy-five percent of the patients in this series maintained adequate medialization for 2 to 3 months, and some patients maintained excellent long-term voice quality. The variability in the duration of medialization may be attributed to a number of factors. Since the resorption rate of injected fat at various sites in the head and neck ranges from 20% to 90%, one would also expect various degrees of resorption in the larynx, and therefore a large variation in the duration of medialization. Progressive muscle atrophy from chronic denervation may also be a factor in the progressive loss of bulk. Canine studies have shown that atrophy in denervated laryngeal muscles can occur as early as 4 to 6 weeks.[10] It is unlikely that reinnervation occurred in many of our patients — certainly not in those who had extirpation of extensive skull base tumors, in the patient with terminal lung cancer, or (probably) in some of the patients with penetrating neck trauma, in whom the recurrent or vagus nerve may have been transected. Such lack of reinnervation as well as some degree of resorption of the fat would explain why only a grade II voice was achieved in many of our patients long-term. It appears that many of these patients who would not be expected to have reinnervation experienced slight breathiness between 2 and 3 months after injection and maintained the same quality of voice thereafter. On the other hand, some of the patients with a good prognosis for reinnervation — ie, idiopathic, carotid endarterectomy, or intubation — maintained an excellent voice beyond 6 months. It is possible that these patients had partial reinnervation without return of vocal fold motion, because of either laryngeal synkinesis and/or cricoarytenoid joint fibrosis. That the muscle bulk would be maintained with reinnervation would explain the good long-term results attained in these patients.

Although we recognize that objective measures are important in evaluating the efficacy of phonosurgical procedures, we have not found these measures, such as maximum phonation time (MPT), to accurately reflect the degree of glottic competence in patients with unilateral vocal fold paralysis. Subjectively, many of our patients experienced reduced vocal effort, but demonstrated only a slight improvement in MPT postoperatively. This may be a reflection of the patients' attempt to compensate for the breathy hypophonia by forced supraglottic hyperactivity during the preoperative evaluation, which would result in an artificially high MPT. Another factor affecting MPT is pulmonary function. The MPT will be reduced in patients with poor pulmonary function, such as in patients with lung cancer and elderly patients, and again may not accurately reflect the degree of glottic competency. For these reasons, the efficacy of medialization with fat injection was assessed by subjective evaluation and sequential MRI imaging. The findings on MRI indicated that the injected fat does survive up to 6 months; however, the signal intensity decreased after 3 months. This suggests that some resorption does occur over time. Unfortunately, it was not possible to quantitate the volumetric loss. Such volumetric loss has been previously demonstrated in animal studies. The long-term survival of injected autologous fat in the thyroarytenoid muscle has been studied histologically in the canine model by Zaretsky et al,[11] as well as Mikus et al.[12] These histologic studies showed presence of the injected fat in the muscle beyond 3 months; however, resorption did occur with time. In the study by Zaretsky et al, persistence of injected fat was seen in the thyroarytenoid muscle at 9 months, although the authors did note progressive loss of medialization on endoscopic examinations, suggesting that some of the injected fat does resorb with time. Similar findings were also reported by Mikus et al, who found the fat graft survival rate was 22% to 31% at 6 weeks and 10% to 21% at 12 weeks.

The method of fat harvesting and processing can influence survival of the fat cells. Controversy exists in the literature regarding the optimal technique. Mikus et al[12] compared fat survival rates for the technique of liposuction versus that of purification by morselization, centrifugation, and aspiration of "pure" fat cells. They found that the survival percentages were slightly better with the liposuction technique. In the present study, the fat was harvested through an abdominal incision, morselized, and washed thoroughly of blood and connective tissue debris in all but 3 patients. This technique was used because we felt that liposuction would be more likely to damage the adipose cells. Whether this technique yields longer survival of the adipose cells compared to liposuction or the purification technique developed by Mikus et al is not known at this time. Since the number of patients in the liposuction group is very small in our study, conclusions regarding the safety or success of the procedure related to the method of fat harvesting cannot be drawn. Indeed, further research is needed to evaluate the optimal harvesting and processing methods that would enhance long-term survival of the fat graft.

Autologous fat injection appears to be a relatively safe procedure, as no short- or long-term complications occurred at the injected or donor site in any of the patients. In this human study, histologic evaluation of the injected fat was obviously not possible; however, in a previous study using a canine model, we observed very little inflammatory response to fat that was injected into the thyroarytenoid muscle.[11] It is a relatively safe procedure, provided that the technique is performed properly. The fat should be injected by the same principles used for Teflon injection: it should be injected into the thyroarytenoid muscle at the level of the true vocal fold, and injection into the subglottic space should be avoided. However, in contrast to Teflon injection, fat should be injected to overmedialize the vocal fold well past the midline, as absorption is expected.

CONCLUSIONS

Autologous fat injection for unilateral vocal fold paralysis is a relatively safe and simple procedure with good, predictable results in the first 2 months. The results are less predictable beyond 2 months, as significant resorption can occur. Therefore, unlike Teflon, it is essential that the vocal fold be overmedialized considerably at the time of injection. Our results suggest that autologous fat may be an ideal substance for medialization of a paralyzed vocal fold that is expected to gain return of function. It appears to last longer than Gelfoam, which is currently the only injectable substance used for temporary medialization. It can also be used in cases in which the prognosis of recovery is uncertain, although the patient must be informed that reinjection or another medialization procedure may be necessary after 3 months. Since there are no contraindications or adverse side effects with this technique, injections can be repeated as necessary until the decision is made to proceed with a permanent procedure, such as arytenoid adduction or thyroplasty.

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REFERENCES

1. Kasperbauer JL, Slavit DH, Maragos NE. Teflon granulomas and overinjection of Teflon: a therapeutic challenge for the otorhinolaryngologist. Ann Otol Rhinol Laryngol 1993;102:748-51.

2. Nakayama M, Ford CN, Bless DM. Teflon vocal fold augmentation: failures and management in 28 cases. Otolaryngol Head Neck Surg 1993;109:493-8.

3. Schramm VL, May M, Lavorato AS. Gelfoam paste injection for vocal fold paralysis: temporary rehabilitation of glottic incompetence. Laryngoscope 1978;88:1268-73.

4. Ford CN, Bless DM. Clinical experience with injectable collagen for vocal fold augmentation. Laryngoscope 1986;96:863-9.

5. Koufman JA, Isaacson G. Laryngoplastic phonosurgery. Otolaryngol Clin North Am 1991;24:1151-77.

6. Crumley RL. Update: ansa cervicalis to recurrent laryngeal nerve anastomosis for unilateral laryngeal paralysis. Laryngoscope 1991;101:384-8.

7. Mikaelian DO, Lowry LD, Sataloff RT. Lipoinjection for unilateral vocal cord paralysis. Laryngoscope 1991;101:465-8.

8. Brandenburg JH, Kirkham W, Koschkee D. Vocal cord augmentation with autogenous fat. Laryngoscope 1992;102:495-500.

9. Boyce RG, Nuss DW, Kluka EA. The use of autogenous fat, fascia, and nonvascularized muscle grafts in the head and neck. Otolaryngol Clin North Am 1994;27:39-68.

10. Shindo MS, Herzon GD, Hanson DG, et al. Effects of denervation on laryngeal muscles: a canine model. Laryngoscope 1992;102:663-9.

11. Zaretsky LS, Shindo ML, deTar M, Rice DH. Autologous fat injection for vocal fold paralysis: long-term histologic evaluation. Ann Otol Rhinol Laryngol 1995;104:1-4.

12. Mikus JL, Koufman JA, Kilpatrick SE. Fate of liposuctioned and purified autologous fat injections in the canine vocal fold. Laryngoscope 1995;105:17-22.

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TREATMENT OF CHRONIC PARANASAL SINUS PAIN WITH MINIMAL SINUS DISEASE

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A common problem for otolaryngologists are patients who present with recurrent, persistent sinus pain that appears out of proportion to the findings on physical examination. Often these patients have a history of recurrent sinusitis that required antibiotics or surgical intervention. Many have had repeated surgical procedures because of this pain. Other common past medical histories may include allergic rhinitis, facial trauma, or dental disease. Patients who have experienced documented acute sinusitis in the past will often present de novo with similar symptoms, but lack any objective evidence of a new active sinus infection. However, the diagnosis of sinusitis is not clearly removed from the patient's or clinician's mind, and the patient is further frustrated by the lack of adequate diagnosis, treatment, and resolution of symptoms. These patients may or may not be experiencing an upper respiratory tract infection or allergy with nasal drainage. Often, they are emotionally distraught from recurrent and persistent pain, the lack of resolution of their symptoms, dependency on narcotics and other analgesics, multiple consultations with a variety of clinicians, and the impingement of their symptoms on employment, interpersonal relationships, and societal and family obligations. If sinusitis is not found to be present, the otolaryngologist must help the patient understand this point, reassure him or her that the otolaryngologist will still be vigilant for the development of sinusitis, and refocus the history and workup for some other cause of the recurrent and persistent paranasal pain. We review various treatment approaches to paranasal pains that are not the result of sinusitis.

KEY WORDS — pain, paranasal sinus, sinusitis.

INTRODUCTION

Sinus pain and headache as a result of infection, tumor, and other causes most often resolve with successful medical or surgical treatment of the primary disease process. However, there are many patients referred to us with severe, persistent, and often debilitating sinus, facial, and headache pain who, following a thorough history, physical, and laboratory evaluation, are not found to demonstrate any active disease of the sinuses or other structures of the head, neck, dental, or ocular areas. This presentation focuses on a brief review of relevant basic neuroanatomy and neurophysiology, the adherence to a consistent definition of sinusitis, and the management of patients with a past history of sinusitis with persistent paranasal sinus pain and headache, but with minimal or no obvious residual sinusitis.

NEUROANATOMY AND NEUROPHYSIOLOGY

Peripheral Nervous System Contributors to Pain. Peripheral nerve receptors, called nociceptors, convey information regarding noxious stimuli. There are also low-threshold mechanoreceptive neurons that are stimulated by non-noxious stimuli, and wide-dynamic range neurons that are stimulated by both noxious and non-noxious stimuli.[1,2] In the head and neck, these inputs converge primarily on the trigeminal brain stem sensory nuclear complex, as well as on other cranial nerve nuclei.[3] The sensitivity of the nociceptive afferents increases following even mild injury,[4] resulting in sensitization. Injured tissue releases a variety of chemical irritants, and afferent nerve endings serve as sensors of the chemical and physical environment. This creates a state of hyperalgesia that, coupled with sensitization, results in facilitated nociceptive transmission.

Central Nervous System Contributors to Pain. Afferent stimuli from the periphery undergo processing, including modulation, facilitation, and synapse relaying of the afferent stimuli as the stimuli enter the spinal cord and brain stem.[2,5] It is important to note that there are many peripheral cranial nerve anastomotic interconnections, central cross-synapse of various cranial nerves with other nuclei, and convergence of cranial nerves, cervical nerves, and autonomic nervous system in a broad area of the brain stem.[2] While lateral thalamic projections are associated with discrimination of location regarding stimulus origin, the medial thalamic projections play an important role in the emotional and perceptual responses to head and neck pains. All these factors may help explain the referral patterns of pain, extension of pain from one area to another, development of myofascial pain, various forms of vascular headaches, muscle contraction headache, a variety of neuropathic pains, and exacerbation of pain symptoms with emotional

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stress.[6-8]

Plasticity and Deafferentation. Plasticity and deafferentation may be possible explanations for some presentations with persistent paranasal sinus pain, pressure, and headache in patients with a history of trauma, repeated surgical procedures, dental extractions, turbinectomies, chronic allergies, or repeated infections who now present without any obvious disease.

A characteristic being recognized by a number of researchers, termed plasticity, refers to the results of certain initiating noxious injuries whereby the nervous system's ability to handle information is altered, resulting in that information's being confused with other neural signals. In the case of pain, stimuli that would normally be perceived as nonpainful are, as a result of plastic changes in the nervous system, perceived as painful. Alterations in the afferent input can result in chemical and morphologic changes within the central nervous system. Plasticity may result from injury or trauma. These changes can result in a number of conditions such as causalgia, neuralgia, and other neuropathic conditions.[9-11] As stated earlier, the release of various neurochemical mediators involved in inflammation can result in heightened sensitivity, and the spread of pain and inflammation to adjacent tissues. There are studies that have also demonstrated heightened excitability of central neurons in nociceptive pathways that can be explained by a central enhancement of the convergent afferent inputs to the more central neurons. Such effects and changes may occur within minutes or, more commonly, over time, in situations of prolonged trauma, repeated trauma, or inflammation.

Deafferentation refers to the destruction, damage, avulsion, or transection of nerve tissue. Situations that may result in modified nociceptive transmission and processing include abnormal firing patterns in nerves after demyelination, nerve transection resulting in neuroma formation, and nerve damage with abnormal central modulation and inhibition following deafferentation. Pain of this type can also be seen in some individuals following dental extraction resulting in the phenomenon of "phantom tooth pain."

Therefore, given the studies to date, it is reasonable to speculate that plasticity and deafferentation may occur, involving the trigeminal somatosensory system, following infections and surgeries of the sinuses, dental extractions, and turbinate resection, to mention just a few possibilities. There may be central neural changes that occur after trauma or routine therapeutic procedures in some individuals that result in deafferentation, and the likelihood that these changes may contribute to the development of chronic pain.[2,10]

Segmental and Central Modulation of Pain. Modulation of somatic and visceral afferent inputs can also occur at the segmental level.[2,12] Noxious stimuli can be suppressed by non-noxious stimuli, such as through the use of transcutaneous electrical nerve stimulation or acupuncture. This type of neuronal inhibitory control has been termed diffuse noxious inhibitory control.[2,13-15] In addition, there are central controls involving enkephalins, endorphins, dynorphin, serotonin, noradrenaline, and other neurochemicals. These endogenous substances may activate descending control systems or be involved in their actions. Stimulation of the midbrain periaqueductal gray matter and the nucleus raphe magnus suppresses the activity of nociceptive trigeminal brain stem neurons, as well as associated behavioral responses and reflexes.[2,13]

Migraine and Tension-Type Headache Pain. The pathogenesis of migraine headache is still not entirely clear. For decades, it has been considered a vascular disorder, with ergotamine and other vasoconstrictors diminishing extracranial blood flow, pulsation amplitude, and headache pain intensity. Moskowitz[16] has postulated that the trigeminal nerve may be involved in the pathogenesis of migraine. The trigeminal nerve is capable of releasing several neuropeptides, and activation of the trigeminal nerve in experimental animals leads to plasma protein extravasation in the dura mater.[16] Recently, a more selective cranial vasoconstrictor, sumatriptan succinate, which works at the specific 5-HT1D (5-hydroxytryptamine receptor 1 subtype D) receptor site, has been found effective in the treatment of migraine.[17] Sumatriptan has an action in antagonizing the extravasation in the dura mater produced by trigeminal nerve stimulation, and this may explain in some part its effectiveness in providing relief during a migraine attack.[16] It is interesting to note that many patients who feel they have recurrent sinusitis, but lack evidence of a current sinusitis, experience decreased paranasal head pain with the use of various nasal spray vasoconstrictors. This may be a diagnostic clue that the patient has a vascular headache pain.

For several decades, tension-type headaches have been associated with extracranial and nuchal muscle contracture. There are investigators who have considered a combination of vascular disturbances mediated by the trigeminal and cervical nerves in combination with increased muscle contraction as causes for the symptoms of tension-type headache.[16]

TREATMENT OF PARANASAL SINUS PAIN

The focus of this presentation is on the treatment of patients who present with chronic paranasal sinus pain and a past history of documented sinus disease, but who now present with no obvious current disease to account for their pain.

Definition of Sinusitis. A significant problem in the diagnosis and treatment of sinusitis is the lack of precision and specificity by clinicians in the diagnosis of sinusitis. In our clinical experience, we define sinusitis as an upper respiratory tract infection with purulent discharge from the nasal passages or nasopharynx; intermittent or persistent fever; intermittent or persistent facial pain, tenderness, or headache over the areas of the maxillary, ethmoid, or frontal sinuses; supporting laboratory documentation of abundant white cells in the nasal discharge; culture of the discharge indicating an abundance of a particular organism or organisms; and radiographic evidence of opacification or air fluid levels on Caldwell views, Waters views, or computed tomography scans. The distinction between acute and chronic sinusitis is arbitrary and relates to the time duration of the disease process, and expected resolution of an acute infection with an appropriate course of antibiotic therapy.

In our experience with a large number of patients without any tangible evidence of sinusitis, one of the most important services an otolaryngologist can perform for patients presenting in this manner is to carefully document the lack of any evidence of sinusitis, and clearly communicate this fact to the patient. Where there is evidence of infection, clearly indicating sinusitis, this should be treated appropriately. If pain persists, this fact should aid in subsequent diagnosis and treatment.

The pain of hypersensitivity reactions, vascular headaches including migraine and cluster headaches, tension-type headaches, neuralgias, temporomandibular joint disorder, myofascial pain, infection, allergies, facial trauma, tumors, dental disease, and ocular disease may occur once, repeatedly, or chronically and result in chronic pain. The treatment for these seemingly diverse acute and chronic disorders shares many of the same basic science considerations regarding neuroanatomy and neurophysiology outlined above.

Headache Disorder. Following a careful evaluation of sinus disease in a patient with symptoms emanating from the sinus area, but without evidence of sinus disease, the patient may be concluded to have migraine headaches, or a related headache type. As sinusitis and other intracranial structural abnormalities are excluded through history, imaging, and other diagnostic means, it is necessary at this point to redirect questions toward a more targeted history. This should not necessarily be viewed as evidence of a poor initial history. Quite often, it has been the patient or referring physician influencing the presentation as a sinusitis.

The evaluation of headaches can be quite time-intensive, and require diagnostic and treatment modalities outside the traditional realm of most specialists. As a result, there are a growing number of physicians from a variety of specialties who are focusing their practice on the diagnosis and treatment of headache disorders. If a vascular headache, tension-type headache, temporomandibular joint disease, trigeminal neuralgia, or other, less common neuralgias of the cranial nerves including postherpetic neuralgia, glossopharyngeal neuralgia, vagal neuralgia, sphenopalatine neuralgia, superior laryngeal neuralgia, or atypical facial neuralgia is apparent, the otolaryngologist has options to manage the patient himself or herself, remain as a consultant with recommendations to the referring physician, or refer the patient to a headache specialist. Regardless of who manages the patient, there is increasing emphasis on a multidisciplinary approach. The more experience a clinician gains in obtaining a history for headache, focusing on pertinent symptoms, the more skilled the clinician will become in diagnosis.

Migraine Headache. Migraine headache is diagnosed by a careful history. Often, in our clinical experience, patients present with a history of paranasal pain and state that decongestants provide relief of the pain. The decongestant is acting as a sympathomimetic vasoconstrictor and, in our opinion, may be providing relief and a clue for an undiagnosed vascular headache, rather than a sinus infection or neuropathic pain.

The use of antibiotics can cause confusion. The use of various antibiotics may only temporally coincide with an otherwise natural resolution of a headache not related to any sinus disease. Indeed, some antibiotics may contribute to headache, and the patient may reason that the wrong initial antibiotic had been chosen, after experiencing a resolution of headache pain temporally associated with a change of antibiotic. Premonitory symptoms or auras and various dietary, emotional, and physical triggers may be evident. Triggers are important considerations in the history. Bright lights, hunger, fasting, skipping meals, hypoglycemia, alcohol, monosodium glutamate, nitrates, tyramine, chocolate, caffeine (or withdrawal from caffeine), vasodilating drugs, changes in barometric pressure, emotional stress, depression, hormonal changes associated with menses, and the excessive use of ergotamine or analgesic abuse with rebound headache are some common precipitants.

Treatment includes nonsteroidal anti-inflamma-

TABLE 1. ABORTIVE AND SYMPTOMATIC THERAPY FOR ACUTE MIGRAINE

- Abortive agents
  - Ergotamine tartrate tablets
    - 2 mg tablet; 1 tablet sublingually at onset, then every 30 minutes as needed; maximum 3 tablets per day, 5 tablets per week
  - Ergotamine tartrate with caffeine tablets
    - 1 mg ergotamine tartrate, 100 mg caffeine per tablet; 2 tablets orally at onset, then 1 tablet every 30 minutes as needed; maximum 6 tablets per day, 10 tablets per week
  - Ergotamine tartrate with caffeine suppositories
    - 2 mg ergotamine, 100 mg caffeine; 1 suppository at onset, then repeat in 1 hour as needed; maximum 2 suppositories per day, 5 suppositories per week
  - Dihydroergotamine mesylate
    - 1 mL intravenously or intramuscularly every hour; maximum 3 mL/d, 6 mL/wk
  - Isometheptene mucate, dichloralphenazone, and acetaminophen capsules (Midrin and others)
    - Isometheptene 65 mg, dichloralphenazone 100 mg, acetaminophen 325 mg; 2 capsules orally at onset, then 1 capsule every hour as needed; maximum 5 capsules per day, 15 capsules per week
  - Sumatriptan succinate
    - 6 mg subcutaneously, then in 1 hour as needed; maximum 12 mg/d
- Nonabortive symptomatic agents
  - Nonsteroidal anti-inflammatory agents
    - Aspirin, ibuprofen
  - Antinausea agents
    - Metoclopramide hydrochloride, prochlorperazine maleate, chlorpromazine hydrochloride
  - Analgesics
    - Acetaminophen, narcotics
  - Nasal decongestants
    - Sympathomimetic nasal sprays

Modified from Elkind and Jaramilla.[18]

TABLE 2. PROPHYLACTIC THERAPY FOR MIGRAINE

β-Adrenergic blocking agents	
Propranolol hydrochloride	80 to 240 mg/d orally in 3 divided doses; start with 20 mg 2 to 4 times per day, and gradually increase as needed and tolerated; may switch to long-acting preparation when stable
Atenolol	50 to 100 mg/d orally in single daily dose
Timolol maleate	20 to 30 mg/d orally in 2 divided doses; start with 10 mg 2 times per day, and gradually increase as needed and tolerated
Nadolol	40 to 120 mg/d in single dose; start with 20 to 40 mg/d, and gradually increase as needed and tolerated
Metoprolol tartrate	100 to 200 mg/d in 2 to 4 divided doses
Calcium channel blocking agents	
Diltiazem hydrochloride	90 to 360 mg/d in 3 or 4 divided doses
Nicardipine hydrochloride	60 mg/d in 3 divided doses
Nifedipine	30 to 120 mg/d in 3 divided doses
Nimodipine	30 mg 3 or 4 times per day
Verapamil hydrochloride	240 to 480 mg/d in 3 divided doses, 1 time per day if sustained-release
Ergot compound	
Methysergide maleate	2 mg/d, increased at 3- to 4-day intervals to 4 to 8 mg/d in divided doses; long-term use associated with retroperitoneal fibrosis

Modified from Elkind and Jaramilla.[18]

tory drugs (NSAIDs), other analgesics, (rarely) opioids, antiemetics, ergotamine tartrate with or without caffeine, and sumatriptan for acute therapy. Important medical histories, potentially severe drug interactions, organ system toxicities, and other severe side effects must be considered, as well as the potential for abuse of various compounds in the control of frequent attacks (Table 1[18]).

Migraine prophylaxis should be considered in individuals who experience migraine attacks more frequently than twice a month and in whom the attacks are easily managed, or in individuals in whom the attacks are so incapacitating, prophylaxis is of clear benefit. Therapy may include the use of beta blockers, calcium channel blockers, tricyclic antidepressants, the newer serotonin reuptake inhibitors (which have fewer side effects than the tricyclic antidepressants), careful use of NSAIDs to avoid the phenomenon of rebound headache, methysergide maleate, biofeedback, and behavioral medicine (Tables 2[18] and 3[18]).

Our approach is conservative: obtaining a careful history, and directing our care toward an elimination diet if indicated, careful use of abortive medications such as NSAIDs to avoid the phenomenon of rebound headache, Midrin, ergotamine, and sumatriptan if indicated. If the headaches occur more frequently than two or three times per month and are considered debilitating, then consideration is given for prophylactic treatment such as with propranolol hydrochloride or atenolol.

Tension-Type Headache. Tension-type headache is the most frequent form of headache. Most often, tension-type headache involves the shoulder and neck muscles, with pain referred to the occipital, parietal, and retroorbital areas, more often developing during the workday, and often lasting for several

TABLE 3. ANTIDEPRESSANT AGENTS

Amitriptyline hydrochloride	25 to 75 mg/d at bedtime or in divided doses; may increase to 200 mg/d
Desipramine hydrochloride	25 to 125 mg/d at bedtime or in divided doses
Doxepin hydrochloride	10 to 25 mg/d at bedtime or in divided doses; may increase to 150 mg/d
Imipramine hydrochloride	30 to 40 mg/d at bedtime or in divided doses; may increase to 150 to 200 mg/d
Nortriptyline hydrochloride	10 to 25 mg/d at bedtime or in divided doses; may increase to 100 mg/d
Fluoxetine hydrochloride (Prozac)	20 mg/d in morning; after several weeks, can increase to 40 mg/d in morning
Sertraline hydrochloride (Zoloft)	50 mg/d in morning; may increase weekly if needed and tolerated to maximum of 200 mg/d
Paroxetine hydrochloride (Paxil)	20 mg/d in morning; may increase weekly by 10 mg/d to 50 mg/d if needed and tolerated

Modified from Elkind and Jaramilla.[18]

days with varying severity. At times the scalp may be extremely sensitive to touch, and the occipital and parietal pains have a bandlike, squeezing quality, without the symptoms of nausea and vomiting. Severe pain may present in a variety of ways, including allodynia of the skin overlying the sinuses or referred to other areas of the facial skin, and variable headache presentations by the same patient, ranging from muscle contraction tension-type headache to vascular-type headache. However, some patients will present with varying severity of tension-type headache that can have more vascular headache features, similar to migraine, rather than myofascial headache pain.

Therapy of acute tension-type headache includes NSAIDs, acetaminophen, and butalbital with or without caffeine, muscle trigger point injections with local anesthetics, muscular injection with botulinum A toxin, and muscular and emotional relaxation techniques including training in biofeedback. A common problem is the chronic overuse and subsequent abuse of acute pharmacologic therapeutic agents resulting in the phenomenon of rebound headaches (Table 4[18]).

TABLE 4. TENSION-TYPE HEADACHE THERAPY

- Acute
  - Pharmacologic
    - Ibuprofen; naproxen sodium; acetaminophen; aspirin; acetaminophen with codeine, butalbital, and caffeine (Fioricet); aspirin with codeine, butalbital, and caffeine (Fiorinal); narcotics
  - Nonpharmacologic
    - Heating pad, warm compresses, hot shower, rest, avoid stress, massage
- Chronic
  - Pharmacologic
    - Antidepressants such as amitriptyline hydrochloride, doxepin hydrochloride, desipramine hydrochloride, fluoxetine hydrochloride (Prozac), and sertraline hydrochloride (Zoloft)
  - Nonpharmacologic
    - Biofeedback, psychotherapy, dietary measures (to reduce vasoactive substances, high caffeine intake resulting in frequent rebound headaches, monosodium glutamate), and physical therapy

Modified from Elkind and Jaramilla.[18]

Our usual approach to therapy for chronic tension-type headache includes careful use of NSAIDs, tricyclic antidepressants, and more recent serotonin reuptake inhibitors; effective training and use of biofeedback; behavioral medicine; and physical therapy (Tables 3 and 4). We will sometimes employ local anesthetic trigger point injections into neck and shoulder muscles with active trigger points in patients with myofascial pain and headaches, in conjunction with physical therapy. We also are injecting botulinum A toxin into facial, neck, and shoulder muscles, along with physical therapy, in individuals who have not responded to the more traditional approaches.[19] The use of botulinum A toxin has been particularly helpful in muscle pain and headaches in individuals with torticollis.[19]

Various Chronic Neuropathic Pains. Trigeminal neuralgia, or tic douloureux, so often presents in such a characteristic manner that it is rarely confused with sinusitis pain, migraine headache, cluster headache, tension-type headache, or temporomandibular joint pain. Trigeminal neuralgia pain is a brief, intense, lancinating unilateral maxillary division pain, accompanied by a facial grimace or facial muscle contracture, triggered by physical stimulation such as cold wind or touch. A refractory period follows the pain attack. Neuralgia can further be classified as posttraumatic neuralgia, if the characteristic neuralgia-type pain began following an antecedent trauma or surgical procedure. It is frequently seen in the trigeminal distribution following dental extraction and involvement of various branches of the first and second divisions of the trigeminal nerve.

Our treatment for trigeminal neuralgia most commonly involves carbamazepine 800 to 1,200 mg/d in divided doses. Monitoring of the complete blood count and drug levels is done regularly, and the dosage is lowered to the minimum level that still maintains relief of symptoms. Serotonin reuptake inhibitors, baclofen, and phenytoin sodium are also used.

Radio frequency lesion, rhizotomy, and microvas-

TABLE 5. THERAPIES FOR NEUROPATHIC HEAD PAIN

α-Adrenergic agonist	
Clonidine hydrochloride (Catapres)	0.1 mg orally 1 or 2 times per day for postherpetic neuralgia, withdrawal symptoms, diarrhea, and migraine, and possibly pain mediated by sympathetic nervous system, such as causalgia or reflex sympathetic dystrophy; more recent nomenclature refers to sympathetic pain as complex regional pain, type I or II
Anticonvulsants	
Carbamazepine (Tegretol)	200 mg 3 times per day for cranial neuralgias; less effective for migraine; serious toxicities are possible; periodic monitoring of drug blood levels, complete blood counts, blood urea nitrogen, and urinalysis has increased safety and efficacy; dosage should be adjusted to needs of individual; low daily initial dosage with gradual increases advised; when adequate control is achieved, dosage may be reduced to minimum effective level
Divalproex sodium (Depakote)	250 mg 1 to 3 times per day
Substance P releaser (substance P is neurotransmitter of pain impluses from periphery to central nervous system)	
Capsaicin (Zostrix)	Apply to area of pain 3 or 4 times per day; avoid contact with eyes; useful in postherpetic neuralgia, diabetic neuropathy, and arthritis, and possibly effective in cluster headaches
Oral form of lidocaine hydrochloride	
Mexiletine hydrochloride	300 mg orally 3 times per day; functions similarly to intravenous lidocaine hydrochloride for variety of neuropathic and vascular head pains
Nonpharmacologic approaches	Behavioral medicine, psychotherapy, physical therapy, transcutaneous electrical nerve stimulation, surgical and chemical neurolysis

Modified from Elkind and Jaramilla.[18]

cular decompression are various techniques employed when medical therapy has failed, or is not a feasible option. However, it is important to firmly establish the diagnosis of trigeminal neuralgia, which has a very distinctive and characteristic presentation. This entity most frequently responds to carbamazepine or radio frequency lesion. Other presentations, such as atypical facial neuralgia, will be less likely to dramatically respond (Tables 3 and 5[18]).

Plasticity and Deafferentation. It is interesting to consider the theoretic possibilities of plasticity and deafferentation, presented earlier in the context of some patients who have undergone repeated sinus surgeries, trauma, turbinectomy, dental extractions, recurrent infections, and allergies and are currently without objective clinical findings consistent with sinusitis. They may still complain of pressure changes while in airplanes, weather pressure changes, and random attacks of pressure and fullness in their sinuses accompanied with sinus headache. They are not responsive to vascular headache medications or decongestants, but do respond to various medications used in neuropathic pain, including tricyclic antidepressants and the newer serotonin uptake inhibitors, antiseizure medications, and the anxiolytic clonazepam. We often prescribe a tricyclic antidepressant such as desipramine hydrochloride, or the newer serotonin uptake inhibitors such as sertraline hydrochloride or fluoxetine hydrochloride, which have fewer side effects. Strong consideration is given to transcutaneous electrical nerve stimulation, behavioral medicine, and substance abuse counseling. Biofeedback techniques may be used as indicated. These forms of treatment may be used simultaneously with subsequent medications for infection and allergies when indicated clinically at future times. When considering neuropathic pain, it is necessary to consider other causes, including nutritional deficiencies, metabolic abnormalities, medication side effects, radiotherapy, chemotherapy, and locally invasive infectious or oncologic disease (Tables 3 and 5).

Post-turbinectomy pain is a rare problem found after turbinectomy, characterized by intense pain on gentle pressure to the mucosa overlying the area of the turbinates. This pain, and a variety of neuropathic pains, may respond variably to cervical sympathetic blockade. Sympathetically mediated pain, now more commonly referred to as complex regional pain types I and II, may play a role in the development and perpetuation of discomfort in post-turbinectomy patients.[20] Exact mechanisms are not known, but sympathetic nerves are known to course with the vasculature of the head and neck.[21] In our experience, some patients report benefit from repeated local anesthetic blocks to the sphenopalatine ganglion, or from repeated cervical sympathetic blocks, with relief lasting well beyond the clinically effective length of time of the local anesthetic. It is unclear whether sympathetic block produces analgesia by 1) interruption of the sympathetic efferents peripherally with a sphenopalatine ganglion block, 2) blockage of afferent fibers traveling in the sympathetic chain with a cervical sympathetic block, or 3) blockage of the afferents in the area of the turbinates, resulting in restoration of normal central nervous system inhibition and modulation in the trigeminal sensory brain stem complex.[2,22,23]

Behavioral Medicine Issues. Frequently, behavioral medicine issues are the focus of treatment. As discussed previously, emotional stress can exacerbate symptoms, and be relevant to the affective-motivational disorder of pain. This may also be particularly important in the level of function ability and disability. Modern multidisciplinary pain treatment often requires behavioral medicine therapy, and biofeedback relaxation techniques to run parallel with other treatments relevant to the patient's particular situation. In our experience, this has met with great success in a variety of headaches, including migraine and tension-type headaches, various neuropathic pains, and a variety of chronic pain situations. Many of the patients with chronic paranasal sinus pain have a certain level of psychosocial dysfunction or disability. The psychosocial dysfunction may include problems with employment, child care, and other family and societal obligations. Frequently, substance abuse and other psychosocial dependency issues develop. Depression, anxiety, neurosis, and compulsive behaviors are frequently noted. It is imperative to consider a behavioral medicine evaluation that can document psychosocial function through standardized tools for assessment. Psychiatric consultation is sometimes indicated. Substance abuse and multiple other psychologic problems are often best addressed in an inpatient pain program facility in which a multidisciplinary approach is available. The patient, family, and referring clinician must be reassured that any of the patient's concurrent organic disease, if present, can still be adequately diagnosed and treated concurrently. For patients with less serious psychosocial impairment, behavioral medicine may still be beneficial regarding relaxation techniques and biofeedback training. Emotional stress may exacerbate a chronic sinus-type pain, and training can often invoke cortical and autonomic influences on modulation and facilitation of the chronic pain state.

SUMMARY

Chronic paranasal sinus pain is best approached in a potentially multidisciplinary way, focused to the individual's needs. It is important to diagnose or rule out sinusitis with consistent criteria. If sinusitis is not found to be present, the patient must understand this point and be reassured that the otolaryngologist will still be vigilant for the development of sinusitis. The physician must then refocus the history and workup for some other cause of the recurrent and persistent paranasal pain. There are many overlapping presentations, but the issues of vascular and nonvascular headache, myofascial pain, atypical facial neuralgia, cranial nerve neuralgias, and other forms of neuropathic pain should be considered early in unusual pain presentations that may include heightened sensitivity, unusual pain patterns, emotional exacerbation, and persistence of pain with resolved or subclinical sinus disease.

REFERENCES

1. Dubner R, Sessle BJ, Storey AT. The neural basis of oral and facial function. New York, NY: Plenum, 1978.
2. Sessle BJ. Neural mechanisms of oral and facial pain. Otolaryngol Clin North Am 1989;22:1059-72.
3. Sessle BJ. The neurobiology of facial and dental pain. Present knowledge, and future directions. J Dent Res 1987;66:962-81.
4. Yaksh TL, Hammond DL. Peripheral and central substrates involved in rostral transmission of nociceptive information. Pain 1982;13:1-85.
5. Sessle BJ, Lucier GE. Functional aspects of the upper respiratory tract and larynx: a review. In: Tiloon JT, Roeder LM, Steinschneider A, eds. Sudden infant death syndrome. New York, NY: Academic Press, 1983:501-29.
6. Sessle BJ, Hu JW, Amano N, Zhong G. Convergence of cutaneous, tooth pulp, visceral, neck, and muscle afferents onto nociceptive and non-nociceptive neurones in trigeminal subnucleus caudalis (medullary dorsal horn) and its implications for referred pain. Pain 1986;27:219-35.
7. Broton JG, Hu JW, Sessle BJ. Effects of temporomandibular joint stimulation on nociceptive and non-nociceptive neurons of the cat's trigeminal subnucleus caudalis (medullary dorsal horn). J Neurophysiol 1988;59:1575-89.
8. Willis WD. The pain system. The neural basis of nociceptive transmission in the mammalian nervous system. Basel, Switzerland: Karger, 1985.
9. Pubols LM, Sessle BJ. Effects of injury on trigeminal and spinal somatosensory systems. New York, NY: Alan R. Liss, 1987.
10. Wall PO. The dorsal horn. In: Wall PO, Melzack R, eds. Textbook of pain. London, England: Churchill-Livingstone, 1984:80-7.
11. Marbach JJ. Is phantom tooth pain a deafferentation (neuropathic) syndrome? Oral Surg Oral Med Oral Pathol 1993; 75:95-105.
12. Young RF, Perryman KM. Pathways for orofacial pain sensation in the trigeminal brainstem nuclear complex of the macaque monkey. J Neurosurg 1984;61:563-8.

13. Dickenson AH, LeBars D. Diffuse noxious inhibitory controls (DNIC) involve trigeminothalamic and spinothalamic neurones in the rat. Exp Brain Res 1983;49:174-80.

14. Fields HL, Besson JM. Pain modulation. Prog Brain Res 1988;77:1-454.

15. Roth N, Schwolow R, Gille HG. Psychophysiological assessment of transcutaneous electric nerve stimulation (TENS) effects upon orofacial pain. Biomed Biochim Acta 1990;49:613-8.

16. Moskowitz MA. Neurobiology of vascular head pain. Ann Neurol 1984;16:157-68.

17. Elkind AH. Mechanisms of head pain. In: Elkind AH, ed. Handbook of headache disorders. Durant, Okla: Essential Medical Information Systems, 1993:24-7.

18. Elkind AH, Jaramilla J. Therapeutic guide for the treatment of headache. Chicago, Ill: National Headache Foundation, 1993.

19. Acquadro MA, Borodic GE. Treatment of myofascial pain with botulinum A toxin. Anesthesiology 1994;80:705-6.

20. Merskey H, Bogduk N, eds. Classification of chronic pain. Descriptions of chronic pain syndromes and definitions of pain terms. 2nd ed. Seattle, Wash: International Association for the Study of Pain Press, 1994:40-3.

21. Hoffman KD, Matthews MA. Comparison of sympathetic neurons in orofacial and upper extremity nerves: implications for causalgia. J Oral Maxillofac Surg 1990;48:720-6.

22. Phero JC, McDonald JS, Green DB, Robins GS. Orofacial pain and other related syndromes. In: Prithvi Raj P, ed. Practical management of pain. 2nd ed. St Louis, Mo: Mosby–Year Book, 1992:237-9.

23. Walsh NE, Ramamurthy S. Neck and upper extremity pain. In: Prithvi Raj P, ed. Practical management of pain. 2nd ed. St Louis, Mo: Mosby–Year Book, 1992:292-5.

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INNER EAR MEDICINE AND SURGERY

The Sixth Symposium and Workshops on Inner Ear Medicine and Surgery, combined with the Prosper Meniere Society Annual Winter Meeting, will be held in Aspen/Snowmass, Colorado, March 22-29, 1997. For further information, contact Prosper Meniere Society, c/o Jane Wells, Meeting Coordinator, 300 E Hampden Ave, Suite 401, Englewood, CO 80110; telephone (303) 788-4235; fax (303) 788-4234.

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AUTHOR'S CORRECTION

The authors of "Aerodigestive Tract Foreign Bodies in the Older Child and Adolescent," April 1996, pages 267-71, have requested that a correction be made in lines 2-3 of the Abstract to the article. The corrected lines should appear as follows:

"A review of patients treated over a 5-year period identified 367 children evaluated for aerodigestive tract foreign bodies, from whom 336 foreign bodies were removed."

Ann Otol Rhinol Laryngol 105:1996

RESIDUE OF MIDDLE EAR CHOLESTEATOMA AFTER INTACT CANAL WALL TYMPANOPLASTY: SURGICAL FINDINGS AT ONE YEAR

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The rate of residual disease after surgery for acquired middle ear cholesteatoma was investigated in 167 ears of 164 patients who had undergone planned second-look tympanoplasty by the intact canal wall technique. Overall, operative findings at the second stage revealed 65 cases of residual disease in 48 ears (29%). These consisted of 50 squamous pearls, 11 cases of the flat, open type, and 4 cases of the extensive type. The configuration of residual disease is closely related to the technical difficulty of eradication, since en bloc removal is much easier in the squamous pearl than in the open or extensive type, mainly because of the unclear margin with the surrounding tissues. The proportion of cases of the open type was greater in children than in adults, in pars tensa cholesteatoma than in pars flaccida cholesteatoma, and in severe primary middle ear disease than in moderate or mild disease, although these differences were not statistically significant. The extensive type occurred in 4 ears with severe primary disease, 3 of which were in children. These results support the value and importance of the staged procedure for middle ear cholesteatoma, particularly when operated on by the intact canal wall technique.

KEY WORDS — middle ear cholesteatoma, residual cholesteatoma, squamous pearl, staged tympanoplasty.

INTRODUCTION

Residual cholesteatoma occurs as a consequence of growth of the fragmental remnant of the matrix inadvertently remaining in the middle ear at the time of cholesteatoma surgery. This happens even after meticulous removal of the matrix under an operating microscope, irrespective of the surgical technique employed.[1-3] Operative findings in revision or second-look operations in our clinic revealed that the residual disease took one of three configurations: a squamous pearl type, a flat, open type, or an extensive type. Configuration of the residue is closely related to the prognosis of this disease, since en bloc removal is much easier with squamous pearls than with the open and extensive types because of their unclear margins. At present, it is not known what is responsible for the formation of the different shapes. The purpose of this study was to investigate the factors pertaining to the incidence of different shapes of residue from the findings at a second-look operation.

SUBJECTS AND METHODS

Clinical data were obtained from 167 ears of 164 patients with acquired cholesteatoma of the middle ear who underwent staged tympanomastoidectomy by the intact canal wall (canal-up) technique during the 15 years from 1980 to 1994. Those ears that had received previous surgery were not included in this study. The patients consisted of 89 males and 75 females, ranging in age from 3 to 70 years. The operation was concluded by either one of the senior surgeons (K.G. and N.Y.).

Surgical procedures basically followed those described by Sheehy and Patterson.[1] In the first stage, after completion of mastoidectomy and posterior hypotympanotomy, the cholesteatoma matrix was removed with care taken to keep the canal wall intact. Efforts were made for complete removal of the matrix by means of a tympanic mirror and/or a rigid endoscope. The incus and the head of the malleus, whenever involved in the disease, were removed to facilitate eradication of the matrix. Then, a silicone rubber sheet was introduced into the tympanic cavity through the facial recess opening and the epitympanum. Perforation of the tympanic membrane was closed by grafting the temporalis fascia with a conventional underlay technique. Ossicular reconstruction for hearing was not attempted at the first stage.

The second-stage operation was conducted 9.9 ± 4.2 months after the first stage, the time span ranging from 4 to 19 months. The incidence of residual disease was evaluated on the basis of the findings at this stage. The χ^2 test, with Yates' correction when necessary, was used for statistical analysis of the data.

CLASSIFICATION OF RESIDUAL DISEASE

Residue of the cholesteatoma was classified into

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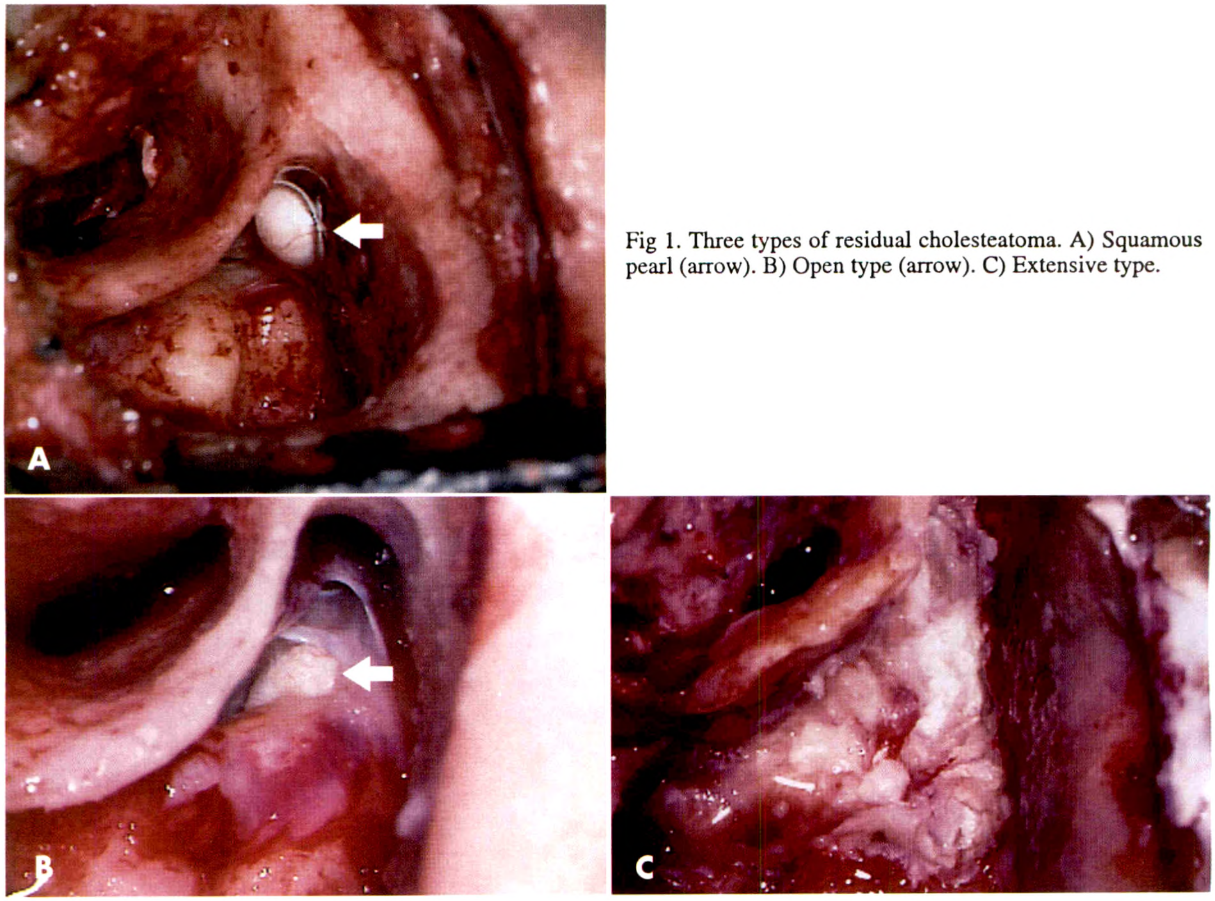

Fig 1. Three types of residual cholesteatoma. A) Squamous pearl (arrow). B) Open type (arrow). C) Extensive type.

three types by configuration: squamous pearl type; flat, open type; and extensive type (Figs 1 and 2). Squamous pearls represent epidermal cysts containing keratin debris inside, and are formed in the middle ear cavity by covering from the regenerated mucous membrane or by burial in the process of granulation. En bloc removal is usually not difficult. The open type of residue is characterized by circumscribed flat epithelium with keratin debris. Detection of this configuration is easy only when keratin remains at its original site. Otherwise, discrimination from regenerated mucosa or scar tissue is often difficult, even under an operating microscope. As the disease is locally confined, careful identification of its extent is important for eradication. The extensive type is defined as residual disease that grows rapidly to involve the middle ear cleft and/or the mastoid cavity extensively. As the residue of this type is thought to be very aggressive in nature,[4] there is a requirement for a third stage or conversion of the surgical procedure from canal-up to the conventional radical operation.

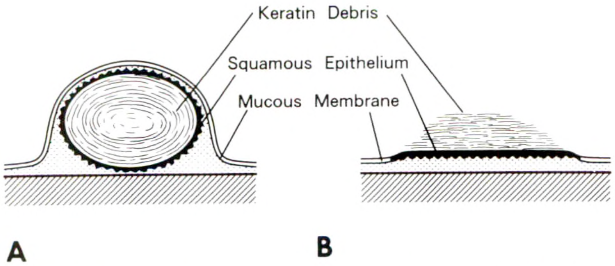

Fig 2. Schematic drawings of A) squamous pearl and B) open type.

RESULTS

Overall, 65 residual cholesteatomas were detected in 48 of 167 ears (29%), consisting of 50 squamous pearl types, 11 open types, and 4 extensive types. Simultaneous occurrence of a squamous pearl and an open-type residue was seen in 3 cases. More than two pearls in 1 ear were found in 9 ears: two pearls in 5 ears, three in 3 ears, and four in 1 ear.

Table 1 shows a comparison between the ages of patients and the detection rates of residual disease at the second stage. Percentages of residual disease decreased with increasing age of the patient: children under 15 showed the greatest incidence, 48%, and adults between 46 and 60, only 8%. There was a trend toward a high proportion of open-type residue in children; however, no substantial difference in the incidence of the three types could be demonstrated between the age groups.

The site of the original cholesteatoma was analyzed with respect to the presence of residual disease.

TABLE 1. FREQUENCY OF RESIDUAL DISEASE BY AGE, SITE, AND SEVERITY

		No. of Ears With Residual Disease				
	No. of Ears	*Squamous Pearl*	*Open Type*	*Pearl & Open*	*Extensive Type*	*Total*
Age (y) at primary operation						
Under 15	42	13	4	0	3	20 (48%)
16 to 30	25	6	1	1	0	8 (32%)
31 to 45	48	10	2	1	1	14 (29%)
46 to 60	37	2	0	1	0	3 (8%)
61 and over	15	2	1	0	0	3 (20%)
Total	167	33	8	3	4	48 (29%)
Site of primary cholesteatoma						
Pars flaccida	73	16	3	0	0	19 (26%)
Pars tensa	77	15	5	2	3	25 (32%)
Unclassified	17	2	0	1	1	4 (24%)
Total	167	33	8	3	4	48 (29%)
Severity of disease at primary operation						
Severe	66	16	5	3	4	28 (42%)
Moderate	52	11	2	0	0	13 (25%)
Mild	49	6	1	0	0	7 (14%)
Total	167	33	8	3	4	48 (29%)

The lesions themselves were classified from the surgical findings as pars flaccida or pars tensa cholesteatomas. When the site of the cholesteatoma was not specified or the lesion was too extensive, a designation of "unclassified" was assigned. As shown in Table 1, the incidence of residual disease, especially that of the open and extensive types, was higher in pars tensa than in pars flaccida cholesteatoma, although this tendency was not statistically significant.

Table 1 also represents the relationship between the severity of middle ear disease at the primary operation and the incidence of residual disease. The severity of disease was classified subjectively as severe, moderate, or mild, on the basis of the state of the cholesteatoma and the middle ear inflammation. Residual disease, especially of the open type, occurred more frequently in cases with severe than moderate or mild primary disease. Simultaneous squamous pearls and open-type residues, and the occurrence of extensive-type residual disease, were recognized only in the severe disease group.

Residual disease tended to develop at locations in which access to the cholesteatoma matrix was difficult at the primary operation (Fig 3). Relationships between the shape and site of the residue are summarized in Table 2. The extensive type, which involved more than two anatomic sites, was not included in this analysis. Residual disease was found more often in the middle ear cleft than in the mastoid cavity. The incidence was the greatest in the attic, especially at the tegmen. The stapes and the tympanic sinus, in-

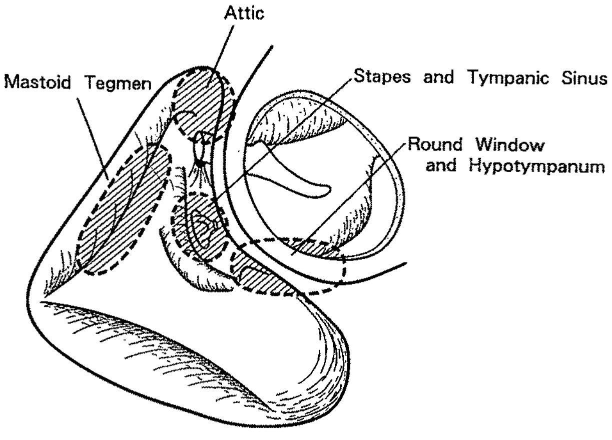

Fig 3. High-risk areas of residual disease after surgery of middle ear cholesteatoma by intact canal wall technique.

TABLE 2. SITE OF INCIDENCE VERSUS SHAPE OF RESIDUAL DISEASE IN 44 EARS

	No. of Residues		
Site of Residue	*Squamous Pearl*	*Open Type*	*Total*
Attic	23	2	25
Stapes and tympanic sinus	17	7	24
Hypotympanum and round window	7	2	9
Mastoid cavity	3	0	3
Total	50	11	61

Data of 4 cases with extensive-type residues were not included.

cluding the horizontal portion of the facial nerve, were also predominant sites of residual disease in cases of pars tensa cholesteatoma. Although the incidence of squamous pearls overwhelmed that of the open type in all sites of the middle ear, 7 of 11 open-type residues were detected at the stapes or the tympanic sinus. Statistical analysis, however, revealed no significant correlation between the shape and the site of the residue.

When residual disease was detected at the second-stage operation, it was carefully removed with the surrounding granulation tissue and the middle ear mucosa. Then, the ossicular chain was reconstructed and the bone defect at the scutum was repaired with a sliced cartilage and bone pate to prevent recurrence. Detailed surgical techniques for reconstruction of the ossicular chain and for prevention of a retraction pocket, together with long-term outcomes of the surgery including hearing results, were reported in our previous papers.[5,6] Two of the extensive cases were subjected to a third-stage operation, and formation of a squamous pearl was indicated in both cases at the later operation. In these 2 cases, surgical procedures were converted from the canal-up procedure to the conventional radical operation.

DISCUSSION

Residual cholesteatoma is among the major causes of failure in surgical treatment of cholesteatoma, the incidence of which varies from 1.5% to 57%[1,2,4,7,8] and depends on a variety of factors such as age of the patient, the site of the original cholesteatoma, the surgeon's skill, and the follow-up period. Residual cholesteatoma may occur long after the initial surgery; the accumulated sum of residual recurrence increases with years after surgery, until it stabilizes at 6 to 8 years postoperatively.[7,8] This indicates that long-term follow-up is most important after surgery for cholesteatoma.

Various factors affecting the incidence of residual disease can be assessed adequately at the second stage of tympanoplasty.[4] It should be noted that we attempted to excise the cholesteatoma matrix as completely as possible during the first-stage operation. Findings at the second stage revealed that residual disease tended to occur, more often than not, in children, in pars tensa cholesteatoma, and in ears with severe primary disease, although these tendencies were not statistically significant, most likely because of the limited sample size. As pointed out by Gristwood and Venables,[7] age at operation was an important prognostic indicator of residual disease. The incidence was the highest in children, and decreased dramatically with increasing age of the patients in this study. Residual disease occurred most frequently in the attic, followed by the stapes and the tympanic sinus. The high incidence in the attic is attributed to the involvement of this region not only in pars flaccida cholesteatoma but also in pars tensa cholesteatoma. The increased incidence at the stapes and the tympanic sinus is explained by the technical difficulty of removing the cholesteatoma matrix completely.

The shape of the residue is considered to represent the state of the cholesteatoma.[4] From a clinical point of view, the squamous pearl is regarded as the end stage because it is easily removed, while an open-type residue retains its potential for further growth. Fortunately, the incidence of squamous pearls is greater than that of the open type. From observations at second-look operations, Schuring et al[4] reported detection of residual disease in 35% of 354 ears, consisting of 21% squamous pearls and 14% open and other types. In their cases, the age of the patients was closely related to the shape of the residue; the ratio of squamous pearls was higher in children than in adults. In the present study, however, squamous pearls occurred more frequently in adults than in children. As a flat cholesteatoma often exhibits more rapid and aggressive growth than pearl-shaped cholesteatomas,[9] the high ratio of open-type residues in children may reflect the insidious nature of cholesteatoma in young patients. The incidence of open-type residues was also remarkable in pars tensa cholesteatoma and in severe primary disease, occurring predominantly around the stapes and the tympanic sinus. However, no firm conclusion concerning the factors responsible for the shapes of the residue can be drawn from the present clinical data.

In an experimental study using rats, Vennix et al[10] reported important findings concerning the shapes of the residues. They transplanted a skin graft in the middle ear of the animals and found that growth of granulation around the retained matrix played an important role in formation of a squamous pearl. They further reported that extensive growth of the graft occurred only in conjunction with bacterial infection.

Residual cholesteatoma occurs even after canal-down tympanoplasty.[1,2,7] However, this problem is more critical in cases treated with the canal-up procedure, since lesions in the attic and the mastoid are obscured until they have progressed quite far. The present study confirmed the previous findings[1,2,7,8] that complete eradication of the cholesteatoma matrix with one surgical procedure is not always possible. Although the surgical procedure should be individualized on a case-by-case basis, we recommend a planned second-look procedure 6 to 12 months after initial surgery maintaining the canal wall, even when the surgeon is fully confident of complete excision of the cholesteatoma. Of prime importance is the need for prolonged postoperative follow-up care.

REFERENCES

1. Sheehy JL, Patterson ME. Intact canal wall tympanoplasty with mastoidectomy. A review of eight years' experience. Laryngoscope 1967;77:1502-42.
2. Sheehy JL, Brackmann DE, Graham MD. Cholesteatoma surgery: residual and recurrent disease. A review of 1,024 cases. Ann Otol Rhinol Laryngol 1977;86:451-62.
3. Smyth GDL. Cholesteatoma surgery: the influence of the canal wall. Laryngoscope 1985;95:92-6.
4. Schuring AG, Lippy WH, Rizer FM, Schuring LT. Staging for cholesteatoma in the child, adolescent, and adult. Ann Otol Rhinol Laryngol 1990;99:256-60.
5. Gyo K, Hinohira Y, Hirata Y, Yanagihara N. Incidence of attic retraction after staged intact canal wall tympanoplasty for middle ear cholesteatoma. Auris Nasus Larynx 1992;19:75-82.
6. Yanagihara N, Gyo K, Sasaki Y, Hinohira Y. Prevention of recurrence of cholesteatoma in intact canal wall tympanoplasty. Am J Otol 1993;14:590-4.
7. Gristwood RE, Venables WN. Factors influencing the probability of residual cholesteatoma. Ann Otol Rhinol Laryngol 1990;99:120-3.
8. Rosenfeld RM, Moura RL, Bluestone CD. Predictors of residual-recurrent cholesteatoma in children. Arch Otolaryngol Head Neck Surg 1992;118:384-91.
9. Schulz P, Bujia J, Holly A, Shilling V, Kastenbauer E. Possible autocrine growth stimulation of cholesteatoma epithelium by transforming growth factor alpha. Am J Otolaryngol 1993;14:82-7.
10. Vennix PPCA, Kuijpers W, Peters TA, Tonnaer ELGM, Ramaekers FCS. Growth and differentiation of meatal skin grafts in the middle ear of the rat. Arch Otolaryngol Head Neck Surg 1994;120:1102-11.

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EPITHELIAL HYPERPLASTIC LESIONS OF THE LARYNX

The International Conference on Epithelial Hyperplastic Lesions of the Larynx will be held October 28-30, 1996, in Ljubljana, Slovenia. For more information, contact Bostjan Luzar, MD, or Aleksandar Vodovnik, MD, Institute of Pathology, Korytkova 2, 61105 Ljubljana, Slovenia; telephone 386 61 1403042 (Int. 268); fax 386 61 301 816; E-mail Vodovnik@IBMI.MF.UNI-LJ.SI.

Ann Otol Rhinol Laryngol 105:1996

ENDONASAL ENDOSCOPIC CLOSURE OF CEREBROSPINAL FLUID FISTULAS AT THE ANTERIOR CRANIAL BASE

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ULRICH GOEDE, MD
MALTE ERIK WIGAND, MD
ERLANGEN, GERMANY

This study reports our indications and limits for endonasal endoscopic closure of dural defects with a cerebrospinal fluid (CSF) leak at the anterior cranial base, and demonstrates our surgical technique. Fifty-three patients with CSF rhinorrhea were reassessed for the success rate of closure of the CSF leak. Surgery was successful in 98%, and 68% of fistulas were closed endoscopically. A free graft of autogenous mucoperiosteum of the inferior turbinate was the most frequently used tissue for defect closure. The endonasal endoscopic route proved relatively safe for the closure of dural tears, irrespective of the cause, up to about 10 × 10 mm. It is characterized by minimal morbidity because of the preservation of sinus ventilation and bony structures, supraorbital nerves, and olfactory fibers. Defects larger in size, predominantly of traumatic origin, were closed via the transfacial approach. The decision on the surgical approach was additionally based on the extent of the facial soft tissue injuries and the localization of the leak.

KEY WORDS — cerebrospinal fluid rhinorrhea, endoscopic surgery.

INTRODUCTION

Cerebrospinal fluid (CSF) rhinorrhea usually is caused by trauma to the head or follows surgery for various inflammatory or tumorous diseases at the anterior cranial base. Rare causes of CSF leaks have been repeatedly described: for example, intracranial tumors, congenital or acquired hydrocephalus, osteomyelitis, meningoencephaloceles, and congenital defects in the osseous anterior cranial base.[1-3] Surgical closure of the CSF leak, irrespective of its pathogenesis, is traditionally performed by the transfacial rhinosurgical route or the subfrontal neurosurgical route.[4-6]

Since the development of endonasal endoscopic surgery in the 1970s by the senior author of this report[7] and Messerklinger,[8] it has proved to be of value in the treatment of the paranasal sinus diseases. Good illumination and magnification offered by the use of an endoscope or a microscope allow the relatively safe exposure of the important regions, including the floor of the anterior cranial fossa. Increased experience with endoscopic surgery led us to expand the surgical indications, and to use this route for the closure of the CSF leaks. The purpose of this study is to report our indications and limits for endonasal endoscopic closure of dural defects at the anterior cranial base, and to demonstrate our surgical technique and results.

PATIENTS AND METHODS

Between 1982 and 1993, 53 patients with CSF rhinorrhea were treated in the department of otorhinolaryngology (Figs 1 and 2). A CSF leak as a complication of paranasal sinus surgery was observed in 24 patients, 20 of whom had received endonasal surgery. A further 24 patients presented with a CSF leak following a minor head injury, and with fracture lines at the floor of the anterior cranial fossa as demonstrated by high-resolution computed tomography. None of these patients required neurosurgical treatment or craniotomy. Five patients, with no history of trauma or previous surgery and no obvious explanation for the CSF rhinorrhea, were considered as having spontaneous fistulas.

Our standard technique for the endoscopic closure of small dural leaks was reported in detail previously.[9] The fistula is inspected with the endoscope, and the integrity of the neighboring bone tested by careful probing with a fine elevator. Mucosal remnants are removed from a surrounding area of 2 to 3 mm with small cupped forceps. An oval mucoperiosteum flap is now harvested from the free edge of the inferior turbinate, carefully smoothed on its internal surface, and freed of bone, but not overly thinned. This free autogenous graft is fixed over the fistula

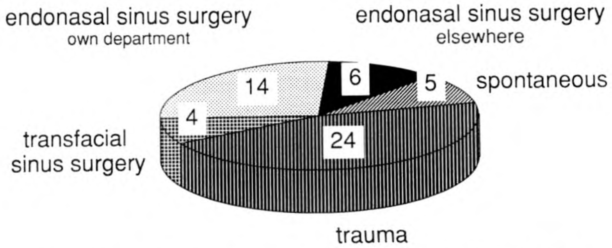

Fig 1. Cause of cerebrospinal fluid leaks in 53 patients.

From the Department of Otorhinolaryngology, University of Erlangen-Nuremberg, Erlangen, Germany.

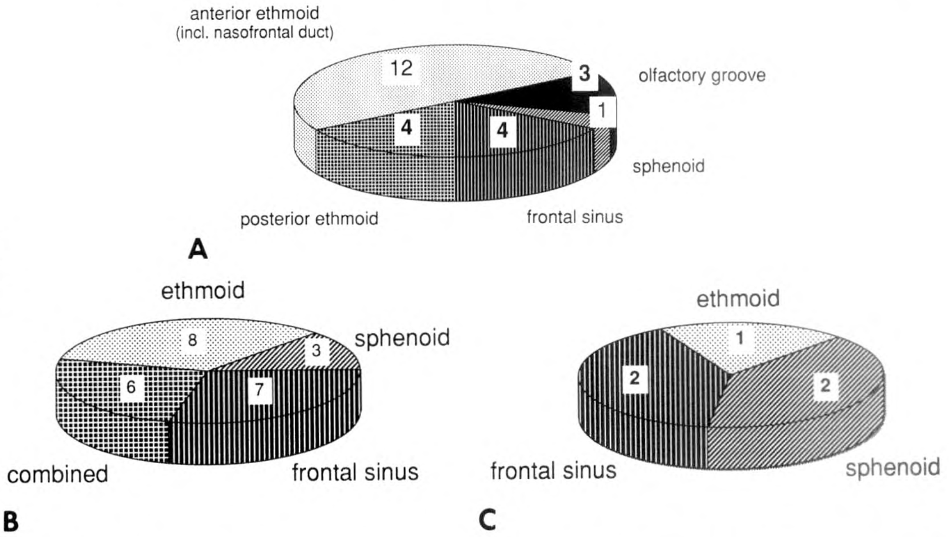

Fig 2. Localization of cerebrospinal fluid fistulas. A) Iatrogenic fistulas (24). B) Traumatic fistulas (24). C) Spontaneous fistulas (5).

with a moderate overlap by means of fibrin glue. The procedure ends with packing of two layers of moist Gelfoam, to be left in place for more than 2 weeks. Under this lies a layer of packing of cotton strips soaked in Aureomycin, exerting slight upward pressure. It should be left in place for 10 days. The patient is additionally put on an antibiotic regimen. Lumbar CSF drainage is not necessary.

In patients not suitable for endoscopic closure of the CSF leak, for reasons discussed below, closure was performed via the classic transfacial route.[10]

Twenty-seven of the 53 treated patients came in for reassessment in the second half of 1994. The examination included history, endoscopy of the nose, and, in selected patients, β2-transferrin detection,[11] intrathecal sodium fluorescein application,[11] and high-resolution computed tomography in coronal sections. Preoperative and postoperative olfactory function was tested by semiquantitative and qualitative clinical olfactometry (squeeze bottle, eight dilutions).[12] The remaining 26 patients were evaluated on the basis of a detailed questionnaire concerning their present status. The average follow-up period was 5.6 years (range 1 to 12 years).

RESULTS

Results are shown in Figs 3 and 4. All 18 iatrogenic fistulas that occurred during sinus surgery in our department were immediately recognized and closed in the course of the same operation. In comparison, of 6 patients operated on elsewhere, in only 1 instance was the fistula recognized intraoperatively and the patient referred for closure. The other 5 patients were referred 3 to 8 weeks after paranasal sinus surgery, either with postoperative meningitis or with rhinorrhea.

Four iatrogenic fistulas that occurred during transfacial frontal sinus surgery were closed by the same route (Fig 3). All but 1 of 20 fistulas complicating endonasal surgery were closed endoscopically. In a patient who developed postoperative meningitis after endonasal sinus surgery, a 2-cm large bony defect of the ethmoidal roof was closed by the transfacial approach.

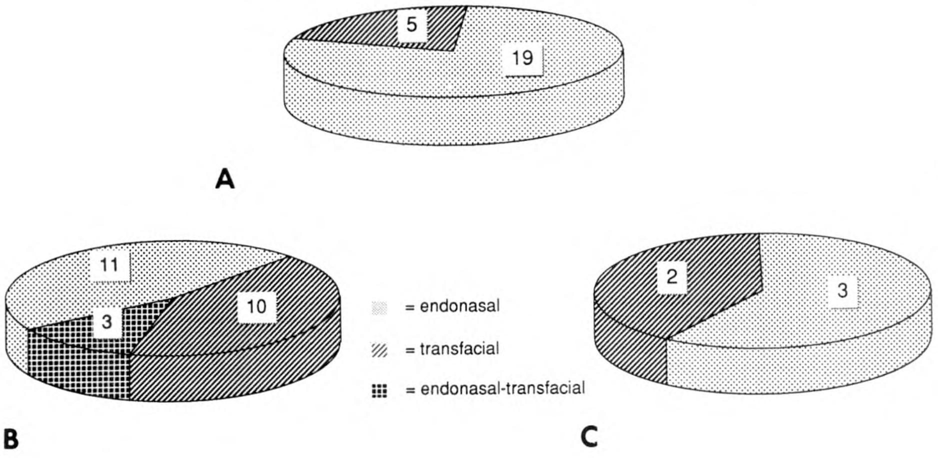

Fig 3. Surgical approaches for closure of fistulas. A) Iatrogenic fistulas (24). B) Traumatic fistulas (24). C) Spontaneous fistulas (5).

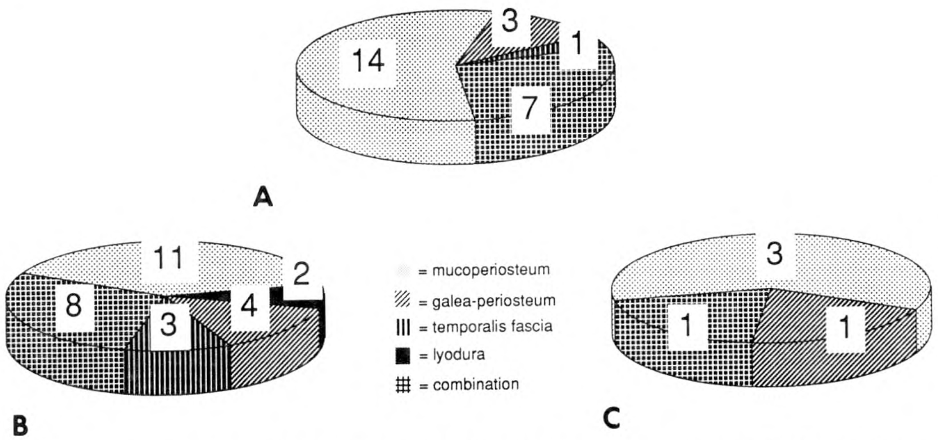

Fig 4. Material used for closure of fistulas. A) Iatrogenic fistulas. B) Traumatic fistulas. C) Spontaneous fistulas.

Three spontaneous CSF leaks, localized in the sphenoid or ethmoid sinuses, were closed endoscopically. Two spontaneous leaks on the posterior wall of the frontal sinus were approached by the transfacial route.

The success rate of permanent closure of the CSF leak was 98%. In 1 patient several endoscopic procedures failed to permanently close the leak, which was then closed by the subfrontal, intradural procedure.

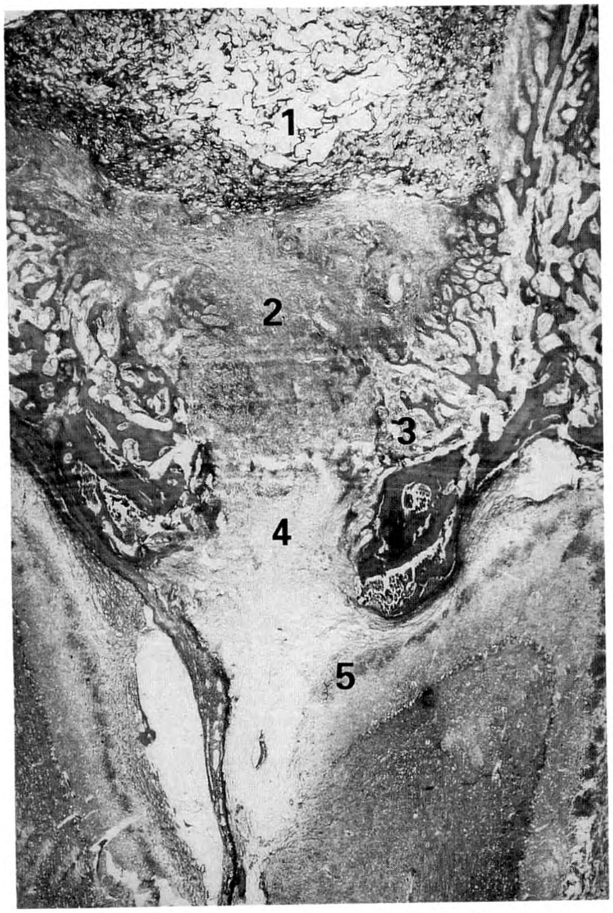

Fig 5. Anterior skull base of rabbit 3 weeks after closure of 4-mm cerebrospinal fluid leak with autogenous free mucosa graft (H & E, original ×5). There is tight adhesion of graft to hypertrophic bone. 1 — Gelfoam, 2 — graft, 3 — new bone formation, 4 — osseous defect at anterior skull base, 5 — endocranium.

DISCUSSION

In contrast to CSF otorrhea, every CSF leak at the anterior cranial base has to be closed surgically to prevent serious complications. The difference lies in the contamination of the nose with pathogens that may cross the interrupted barrier to the brain and lead to meningitis, even many years after the trauma. There are many reports in the literature concerning late complications of persistent CSF rhinorrhea.[4,13] The importance of this problem was confirmed in our present series, in which 6 patients developed complications 3 to 50 years after trauma. Occasionally the rhinorrhea may cease spontaneously because of brain herniation into the dural defect or thin scar formation, but nevertheless, surgery has to be performed.

In a recent extensive review of the literature on the complications of sinus surgery, Lawson[14] listed the incidences of CSF leaks among various reports. The reported range for endoscopic surgery was 0% to 3%. Initial reports of our results were given previously on three occasions.[9,15,16] This study reviews the patient material of the department in an 11-year period with more than 30 surgeons involved. Among a total number of 1,054 endoscopic sinus operations in this period, 14 CSF leaks occurred (1.3%). There were typical regions of danger during the surgery: the lateral lamella of the cribriform plate; the posterior border of the nasofrontal duct; and the olfactory region, including the medial aspect of the middle turbinate. The vast majority of bony injuries and dural tears in the course of endoscopic surgery were only a few millimeters in size, but were readily recognized by the surgeon.

In cases of advanced polyposis it is safer to expose the ethmoidal roof from posterior, beginning at the posterior ethmoid and then advancing along the clearly visible white mucosa of the ethmoidal roof toward the nasofrontal duct. Inadequate exposure of the anterior ethmoid and blind probing of the nasofrontal duct may lead to perforation of the duct's posterior border. Using blunt instruments for polyp removal instead of sharp biting forceps may damage olfactory fibers and produce a CSF leak. The dura is closely adherent to the bone in that region, and tears easily. Also, the placement of a graft over the dural defect but under the bony edges is seldom possible for such small defects.[10] Autogenous mucoperiosteum of the inferior turbinate glued over the fistula offers several advantages. It is readily available in sufficient size for the closure of most defects, only minor surgery for graft harvesting is necessary, and the texture of the mucoperiosteum permits a more secure seal than a simple nasal mucosa flap. Goede et al[17] showed, on the basis of animal experiments and histologic studies, that after the first week the mucoperiosteum tightly adheres to the surrounding bone, and after 3 weeks it is completely transformed into fibrous connective tissue (Fig 5). They suggested that the size of the graft should surpass the defect size by 30%, to compensate for postoperative graft shrinkage.

Furthermore, endoscopic, minimally invasive exposure allowed secure closure of defects localized very close to the ostium of the frontal sinus, and still a ventilated frontal sinus could be preserved. The bony support of the frontal sinus floor, the supraorbital nerves, and the olfactory fibers could thus be preserved, in contrast to the external procedure.

Dural tears up to 15 mm in length could reliably be closed by the aforementioned technique irrespective of their cause. Dural defects up to about 10 × 10 mm could be closed endoscopically by using an additional layer of fascia. Larger defects, predominantly of traumatic origin, were closed via the transfacial approach using temporalis fascia, fascia lata, or galea-periosteum grafts. In addition to the size of the dural defect, the decision about the surgical approach in a traumatic CSF leak depended on the extent of the facial soft tissue injuries and the localization of the leak. Leaks localized in the walls of the sphenoid sinus or the roof of the ethmoids were visualized clearly by the use of an endoscope. In contrast, multiple and transverse fractures of the cribriform plate with or without involvement of the crista galli were better exposed by the transfacial or subfrontal route.

The impairment of olfaction is a well-known complication of transbasal, subfrontal surgery at the anterior cranial fossa.[18] Impairment of olfaction was not observed in our patients after an endoscopic closure of the CSF leak. In patients with traumatic leaks and preserved olfaction, no deterioration of the sense of smell was observed after surgery. The majority of patients with chronic sinusitis with or without polyposis have some degree of impairment of olfaction, which usually improves after the surgery. Deterioration of olfaction due to the surgical procedure was never observed.

REFERENCES

1. Hudson WR, Hughes LA. CSF rhinorrhea: diagnosis and management. South Med J 1975;68:1520-3.

2. Ommaya AK, DiChiro G, Baldwin M, Pennybacker JB. Non-traumatic cerebrospinal fluid rhinorrhea. J Neurol Neurosurg Psychiatry 1968;31:2114-25.

3. Benedict M, Schultz-Coulon HJ. Spontane Rhinoliquorrhoe: Ätiologie, Differentialdiagnose, Therapie. HNO 1991;39:1-7.

4. Grahne B. Traumatic cranionasal fistulas with persistent cerebrospinal fluid rhinorrhoea and their repair with frontal sinus osteoplasty. Acta Otolaryngol (Stockh) 1970;70:392-400.

5. Pirsig W, Limmer P. Der fronto-orbitale Zugang bei der Behandlung frontobasaler Liquorfisteln von Kindern. HNO 1975;23:74-81.

6. Unterberger S. Neuzeitliche Behundlung von Schädelverletzungen mit Beteiligung der fronto- und laterobasalen pneumatischen Räume. Z Laryngol Rhinol 1959;38:441-5.

7. Wigand ME, Steiner W. Endonasale Kieferhöhlenoperation mit endoskopischer Kontrolle. Laryngol Rhinol Otol (Stuttg) 1977;56:421-5.

8. Messerklinger W. Über die Drainage der menschlichen Nasennebenhöhlen unter normalen und pathologischen Bedingungen. 1. Mitteilung. Monatsschr Ohrenheilkd 1966;100:56-68.

9. Wigand ME. Endoscopic surgery of the paranasal sinuses and anterior skull base. New York, NY: G. Thieme, 1990.

10. Denecke HJ, Denecke MU, Draf W, Ey W. Die Operationen an den Nasennebenhöhlen und der angrenzenden Schädelbasis. Berlin, Germany: Springer, 1992.

11. Oberascher G. A modern concept of cerebrospinal fluid diagnosis in oto- and rhinorrhea. Rhinology 1988;26:89-103.

12. Hosemann W, Goertzen W, Wohlleben R, Wolf S, Wigand ME. Olfaction after endoscopic endonasal ethmoidectomy. Am J Rhinol 1993;7:11-5.

13. Boenninghaus HG. Rezidivierende Meningitiden als Folge früherer Schädelbasisbrüche. HNO 1968;16:1-6.

14. Lawson W. The intranasal ethmoidectomy: evolution and an assessment of the procedure. Laryngoscope 1994;104(suppl 64).

15. Wigand ME, Hosemann WG. Microsurgical treatment of recurrent nasal polyposis. Rhinol Suppl 1989(suppl 8):25-30.

16. Hosemann WG, Nitsche N, Rettinger G, Wigand ME. Die endonasale, endoskopisch kontrollierte Versorgung von Duradefekten der Rhinobasis. Laryngol Rhinol Otol (Stuttg) 1991;70:115-9.

17. Goede U, Hosemann WG, Sauer M, Wigand ME. Autologe freie Nasenmuscheltransplantate an der Frontobasis — histologische und klinische Untersuchungen. Eur Arch Otorhinolaryngol Suppl 1992(suppl 2):114.

18. Spetzler RF, Herman JM, Beals S, Joganic E, Milligan J. Preservation of olfaction in anterior craniofacial approaches. J Neurosurg 1993;79:48-52.

Ann Otol Rhinol Laryngol 105:1996

MICROBIOLOGY OF TRACHEAL GRANULATION TISSUE ASSOCIATED WITH SILICONE AIRWAY PROSTHESES

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Granulation tissue formation and contraction is inevitable during the healing of open wounds. The presence of infection and a foreign body, such as a silicone airway prosthesis or other tracheotomy tube, magnifies this response. In airway reconstruction, wound contraction secondary to chronic inflammation is a liability. Cultures of granulation tissue were taken from 12 patients with silicone cannulas of T-Tubes. Polymicrobial colonization predominated, with variable combinations of gram-positive, gram-negative, and anaerobic organisms being present. On the basis of sensitivity data, amoxicillin–clavulanate potassium and ciprofloxacin hydrochloride were chosen as empirical antimicrobials. Anecdotally, impressive decreases in the quantity of granulation tissue were seen. We recommend a prospective trial to more accurately define the role of antibiotics in this setting.

KEY WORDS — airway prostheses, granulation tissue, microbiology, silicone, tracheotomy tube.

INTRODUCTION

Granulation tissue is an integral part of the wound healing process. Histologically, it consists of fibroblasts, macrophages, loose connective tissue, and newly formed capillaries that function as a unit to remove debris, improve delivery of oxygen, and deposit fibroblasts. As the granulation moves into the open wound, the fibroblasts take on the ultrastructural and functional characteristics of smooth muscle. Now called myofibroblasts, they become contractile and act in a concerted manner to pull the edges of the wound centripetally, decreasing the area of the scar.[1]

In the surgical wound that has been sutured, the formation of granulation is minimized, as clean wound edges are apposed and primary healing may occur. However, in airway reconstruction there are often areas of open wound that must mucosalize prior to healing. Granulation tissue in the open wound must be minimized, as contraction of the wound will lead to narrowing of the lumen and, thus, a compromised airway.[2] Additionally, the granulations themselves can be a barrier to airflow and inhibit decannulation.

The presence of granulation tissue associated with tracheotomy tubes is well known and has been described as a common complication in the pediatric otolaryngologic literature.[3] In fact, giant suprastomal granulomas have been reported to present as laryngeal masses requiring surgical removal.[4] Granulation tissue in this setting is considered to be the response to a low-grade infection that is associated with a foreign body, the tracheotomy tube.[5]

The bacteriology of tracheal granulation tissue associated with pediatric airway reconstructions and Teflon stents has been reported. In 19 patients, 57 different bacterial isolates were grown with aerobic cultures. The most common gram-positive organisms were various *Streptococcus* species, accounting for 21 of 30 isolates. *Pseudomonas aeruginosa* was the most common gram-negative organism, accounting for 9 of 24 isolates. Three patients' cultures grew *Candida albicans*. All cultures but 1 were polymicrobial.[5] Grillo et al[6] described the microbiologic findings of cultures taken from open stomas and granulations at anastomotic sites in tracheal reconstructions. *Pseudomonas aeruginosa* and *Staphylococcus aureus* were the most common isolates. This granulation tissue was associated with the use of nonabsorbable suture (foreign body), and not with an active clinical infection. When polyglycolic acid suture material was used, the granulations were no longer a serious problem.

The purpose of this study is to define the bacteriology of granulation tissue associated with silicone airway prostheses, namely, the Montgomery cannula and Safe T-Tube (Boston Medical Products, Inc, Westborough, Mass). As previously mentioned, excessive granulation tissue at the site of repair after laryngotracheoplasty has a potentially deleterious effect on healing, with resultant scar contracture and a decreased airway lumen. In addition, patients with long-term T-Tubes or tracheotomy cannulas are significantly inconvenienced by the bleeding and obstruction that may occur because of granulation at the tracheotomy site. If appropriate antibiotics are utilized, these granulations can be avoided, with resultant improvement in surgical results and patients'

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TABLE 1. PATIENT DATA

Patient	*Age*	*Sex*	*Diagnosis*	*Prosthesis*
1	29	F	Congenital subglottic stenosis	T-Tube
2	59	M	Obstructive sleep apnea	Cannula
3	27	F	Acquired subglottic stenosis, bilateral true vocal cord paralysis	Cannula
4	25	F	Acquired subglottic stenosis	T-Tube
5	42	M	Acquired subglottic stenosis, posterior glottic stenosis, bilateral true vocal cord fixation	Cannula
6	78	M	Acquired subglottic stenosis	T-Tube
7	54	M	Obstructive sleep apnea	Cannula
8	69	F	Tracheotomy, head and neck cancer	Cannula
9	25	M	Supraglottic bullous pemphigoid	Cannula
10	68	M	Bilateral true vocal cord paralysis of unknown cause	Cannula
11	23	M	Acquired subglottic stenosis	T-Tube
12	39	M	Obstructive sleep apnea	Cannula

satisfaction with their prostheses.

MATERIALS AND METHODS

Twelve patients were identified over a 14-month period who had significant granulation tissue associated with either a Montgomery tracheotomy cannula or a T-Tube (Table 1). Most patients had cannulas as treatment for either obstructive sleep apnea or a transitional airway guard on the way to decannulation after laryngotracheoplasty. The remaining patients had a T-Tube either permanently or as part of their surgical plan for airway reconstruction.

The granulations were sent from the office or operating room on aerobic and anaerobic swabs for the appropriate cultures. The tissue was ground and for aerobic cultures plated on blood agar, MacConkey media, and meat broth and grown at 35°C in 5% to 10% carbon dioxide. Anaerobic cultures were plated on CDC-Laked SB with KV media, as well as CDC-anaerobic blood agar, also at 35°C (atmosphere 85% nitrogen, 10% carbon dioxide, 5% hydrogen). Antibiotic sensitivities were determined by standard minimal inhibitory concentration measures for blood levels of the drugs utilizing the microdilution method. All *Streptococcus* species are considered sensitive to penicillin and therefore are not usually tested against other antibiotics. Anaerobic organisms are typically not analyzed for antibiotic susceptibility, either. Therefore, *S aureus* and gram-negative rods are the only bacteria whose susceptibilities were consistently reported.

RESULTS

Out of 12 patients and 12 separate cultures of tracheal and stomal granulations, 30 separate bacterial isolates were seen, averaging 2.5 isolates per patient. Seventy-five percent had polymicrobial cultures, ranging from 2 to 5 bacteria per patient. *Staphylococcus aureus* and *P aeruginosa* were the most commonly found organisms, being present in 6 isolates each. Seventy-five percent of the patients had gram-positive organisms, while 50% had gram-negative rods in their cultures. There were 5 anaerobic

TABLE 2. GRANULATION TISSUE CULTURE DATA

Patient	Staphylococcus *sp*	Streptococcus *sp or* Diphtheroid *sp*	Candida *sp*	Pseudomonas	Proteus	Escherichia coli	*Anaerobes*
1	X	XX					
2	X				X		X
3	X			X			
4				XXX	XX		
5		X					X
6				X			
7		X				X	X
8	XX						
9	X						
10	XX						
11			X				
12		XX		X			XX

X — single bacterial isolate.

TABLE 3. ANTIMICROBIAL SUSCEPTIBILITY ON GRANULATION TISSUE CULTURE

	Streptococcus aureus (%)	Pseudomonas aeruginosa (%)	Proteus mirabilis (%)	Escherichia coli (%)
Ampicillin sodium	0		33	100
Amoxicillin–clavulanate potassium	83			
Clindamycin phosphate	83			
Cefazolin sodium	83		33	100
Ceftazidime		50		
Ceftriaxone sodium		17		
Ciprofloxacin hydrochloride		50	33	100
Erythromycin	50			
Gentamicin sulfate	100	67	66	100
Oxacillin sodium	83			
Penicillin G potassium	0			
Trimethoprim-sulfamethoxazole	100			
Tetracycline hydrochloride	67			
Vancomycin hydrochloride	100			

bacteria isolated in 4 different patients. One patient had *Candida* as his only isolate (Table 2).

The sensitivities of the isolated *S aureus* and gram-negative rods were determined (Table 3). Oral medications to which the gram-positive bacteria were reasonably susceptible included amoxicillin–clavulanate potassium, clindamycin phosphate, oxacillin sodium, and trimethoprim-sulfamethoxazole. The only oral antibiotic that had activity against the gram-negative rods was ciprofloxacin hydrochloride.

DISCUSSION

The use of silicone T-Tubes and cannulas has been well described in the management of obstructive sleep apnea and stenoses of the larynx, subglottis, and trachea.[7] In laryngotracheoplasty, the T-Tube serves the dual purpose of acting as a stent for the new lumen and maintaining an airway during the healing process. These silicone prostheses act as foreign bodies, inciting infection and granulation tissue formation. This granulation may result in contracture of the wound and thereby decrease the lumen size.

The amount of scar tissue in a wound is greatly influenced by the inflammatory response that the injury incites. Bacterial contamination of a wound increases this inflammation. A foreign body such as a tracheotomy tube can act as a source of microbial infection. In one study utilizing the injured canine subglottis, bacterial contamination was minimal in the absence of a T-Tube. If a silicone T-Tube was present, the bacterial count was significantly increased. The inflammation due to the infection eventually resulted in worsening of the injury, necrosis of the underlying cartilage, and the formation of granulation tissue. If antibiotics were given in conjunction with the T-Tube, the bacterial counts were kept low. It was concluded that the injury to the subglottis could be minimized in the presence of a T-Tube if bacterial contamination of the stoma could be minimized.[8] A description of the biochemical and histologic properties of tracheal collagen in 2 patients with normal tracheas (at necropsy) and 2 patients with inflammatory tracheal stenosis has been reported. Normal tracheal collagen is predominately type II in the cartilage, and types I and III in the fibroelastic layer. The cartilaginous collagens found in inflammatory stenosis had a higher percentage of types I and III or type II; this finding indicates that the inflammatory reaction had changed the collagen content to one more associated with fibrous tissue.[9] The collagens found in the stenotic airway are of the same types as typically produced by granulation-associated fibroblasts (I and III).[1]

The present bacteriologic findings are consistent with previous reports[4,6] and have prompted us to use antibiotic therapy in order to decrease the granulation tissue response to T-Tubes and cannulas. As a general rule, *Staphylococcus, Streptococcus* species, gram-negative rods, and occasional anaerobes must be covered. Amoxicillin-clavulanate is consistently the best antistaphylococcal medicine in our institution that also covers streptococcal and anaerobic bacteria. The gram-negative rods are typically multi–drug-resistant. The only oral antimicrobial that has reliable activity against *Proteus, Pseudomonas,* and *Escherichia coli* is ciprofloxacin. Therefore, a combination of these two antibiotics has been used to decrease the chronic, low-grade infection that is often associated with indwelling T-Tubes and cannulas. Following laryngotracheoplasty, the patients are treated with this regimen as long as a silicone prosthesis is in the airway — up to 2 months or more. There have been very few antibiotic-associated side effects, such as diarrhea or allergic sensitization. Long-term cannula or T-Tube patients may, on occasion, develop bleeding or obstructing granulations. These are removed in the office or the operating room and the aforementioned antibiotic regimen is begun, to last for 2 to 3 weeks.

Anecdotally, this antibiotic therapy has resulted in a significant decrease in the quantity of granulation seen with T-Tubes after laryngotracheoplasty in the acute setting. In patients with long-term indwelling prostheses, the resolution of the granulation tissue is facilitated by the use of these antibiotics. We feel that these initial findings are important enough to warrant a prospective study to more accurately define the efficacy of antibiotics in this setting.

REFERENCES

1. Clark RAF. Granulation tissue. In: Clark RAF, Henson PM, eds. The molecular and cellular biology of wound repair. New York, NY: Plenum Press, 1988:9, 12, 301, 477.
2. Prescott CAJ. Protocol for management of the interposition cartilage graft laryngotracheoplasty. Ann Otol Rhinol Laryngol 1988;97:239-42.
3. Prescott CAJ. Peristomal complications of paediatric tracheostomy. Int J Pediatr Otorhinolaryngol 1992;23:141-9.
4. Hartig GK, Wiatrak BJ, Myer CM III, Bower CM. Giant suprastomal granuloma as a laryngeal mass. Ann Otol Rhinol Laryngol 1993;102:701-4.
5. Matt BH, Myer CM, Harrison CJ, Reising SF, Cotton RT. Tracheal granulation tissue. Arch Otolaryngol Head Neck Surg 1991;117:538-41.
6. Grillo HC, Zannini P, Michelassi F. Complications of tracheal stenosis. J Thorac Cardiovasc Surg 1986;91:322-8.
7. Montgomery WW. Surgery of the upper respiratory system. Philadelphia, Pa: Lea & Febiger, 1989:365-520.
8. Sasaki CT, Horiuchi M, Koss N. Tracheostomy-related subglottic stenosis: bacteriologic pathogenesis. Laryngoscope 1979;89:857-65.
9. Bellon G, Moreau M, Cam YJ, et al. Modification of collagens in the course of inflammatory tracheal stenoses. Ann Otol Rhinol Laryngol 1985;94:403-8.

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EUROPEAN WORKING GROUP ON GENETICS OF HEARING IMPAIRMENT

The Second HEAR Project Workshop will be held in Milan, Italy, October 11-12, 1996. For further information and submission of abstracts, please contact: Alessandro Martini, MD, Professor of Audiology, Servizio di Audiologia, Clinica ORL, Università degli Studi, Corso Giovecca 203, I-44100 Ferrara, Italy; telephone: +39 532 295429 or 295341; fax: +39 532 295887; E-mail: mma@ ifeuniv.unife.it

Ann Otol Rhinol Laryngol 105:1996

HLA CLASS II–ASSOCIATED GENETIC SUSCEPTIBILITY IN IDIOPATHIC PROGRESSIVE SENSORINEURAL HEARING LOSS

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To investigate the association between genes in the major histocompatibility complex and inner ear disease susceptibility at the DNA level, high-resolution genotyping for HLA class II (HLA-DR, -DQ, -DP) was performed by polymerase chain reaction–sequence-specific oligonucleotide reverse dot blot and polymerase chain reaction–restriction fragment length polymorphism analysis in 34 patients with idiopathic progressive sensorineural hearing loss (PSHL) and in 214 controls. The frequencies of DRB1*0301, DRB3*0101, DQB1*0201, and DPB1*0401 were significantly increased in patients with idiopathic PSHL compared with controls. The DQB1*0301 allele was significantly decreased in the patients. A linkage disequilibrium was probably responsible for the concomitant increase of both DRB1*0301 and DRB3*0101 alleles in patients. The increase of DQB1*0201 in patients was associated with the DRB1*0301 allele. In addition, the telomeric DPB1*0401 allele may act as an independent risk factor. The DQB1*0301 allele may have a protective role in the pathogenesis of idiopathic PSHL. These results suggest that the specific HLA class II gene products may confer susceptibility or resistance to idiopathic PSHL.

KEY WORDS — autoimmune inner ear disease, hearing loss, HLA class II, polymerase chain reaction, restriction fragment length polymorphism, reverse dot blot, sequence-specific oligonucleotide.

INTRODUCTION

The human leukocyte antigen (HLA) class II molecules, HLA-DR, -DQ, and -DP, are highly polymorphic cell membrane–bound heterodimers of α and β glycoprotein chains coded by genes in the HLA or major histocompatibility complex (MHC) on the short arm of chromosome 6. The HLA class II molecules play an important role in the function of the immune system by presenting processed peptide antigens to $CD4^+$ T cells. They are involved in the selection and activation of $CD4^+$ T cells, which regulate both humoral and cell-mediated immune responses to protein antigens. Therefore, it is conceivable that the susceptibility to many supposedly autoimmune diseases is influenced by the inheritance of the HLA genes. Much work has been done on the polymorphic class II loci HLA-DR, -DQ, and -DP in autoimmune disease. Genetic predisposition to over 40 human diseases has been associated with specific class II HLA alleles.[1]

It is now believed that autoimmunity is associated with the pathogenesis of some of the previously ill-defined inner ear diseases, including progressive sensorineural hearing loss (PSHL), sudden deafness, Meniere's disease, and otosclerosis,[2-5] since autoimmune inner ear disease was first described as a distinct clinical entity by McCabe[6] in 1979. By the HLA serologic typing method, using panels of antibodies to identify different alleles, several attempts have been made to correlate HLA phenotypes and inner ear disease.[7,8] These studies have shown that inner ear disease susceptibility is associated with the MHC, although the exact nature of the association remains controversial.

With the development of the polymerase chain reaction (PCR) technique and its application in HLA typing, it has been found that a serologically defined specificity may actually be composed of a family of related HLA alleles that differ slightly from one another in their polymorphic residues. In many autoimmune diseases, the HLA molecules with a higher frequency differ only in the peptide-binding clefts of the first protein domain from the other HLA molecules that are not disease-associated. These variations can only be detected by molecular methods. Moreover, more detailed molecular studies of HLA genes in patients with autoimmunity have shown that some HLA-disease associations were much stronger than they appeared to be when calculations were based on less precise serologic typing.

In order to investigate the HLA alleles that may contribute to the genetic susceptibility to idiopathic

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TABLE 1. PCR PRIMERS USED FOR HLA-DRB AND -DPB PCR-RDB TYPING

Specificity	*Name*	*Codons*	*Sequence*	*Product Size (bp)*
DRB (B1 + B3 + B4 + B5)	DRB-1	−7 to 2	GATCCTTCGTGTCCCCACAGCACG	197
	DRB-2	87 to 94	GCCGCTGCACTGTGAAGCTCTC	
DR4	DR4-1	5 to 13	CCACGTTTCTTGGAGCAGGTTAAAC	178
	DRB-2	87 to 94	GCCGCTGCACTGTGAAGCTCTC	
DPB1	DPB-1	−4 to 7	CCTCCCCGCAGAGAATTAC	185
	DPB-2	87 to 93	CTCGGCGCTGCAGGGTCA	

PCR — polymerase chain reaction, RDB — reverse dot blot, bp — base pair.

PSHL at the DNA level, highly discriminatory HLA genotyping for HLA-DR, -DQ, and -DP alleles was performed in the patients by means of the PCR–sequence-specific oligonucleotide (SSO) reverse dot blot technique and PCR–restriction fragment length polymorphism (RFLP) analysis. We attempted to assess whether DR and DQ types are indeed associated with idiopathic PSHL, and whether DP alleles also contribute to susceptibility, as the genes of DP have not previously been examined in this condition.

MATERIALS AND METHODS

Subjects. Thirty-four unrelated white patients with idiopathic PSHL, living in Belgium, were included in this study. These patients, from 20 to 62 years old (average 41 years), consisted of 18 men and 16 women. A precise medical history was obtained from the patients. None of them suffered from metabolic diseases, hereditary diseases, or any other diagnosed autoimmune diseases. None of them had a positive family history for autoimmune disease. Two patients had a slight prodrome of viral infections. The hearing loss of all patients manifested as greater than 30 dB at any frequency. Progression of hearing loss was defined as a greater than 10-dB threshold shift at any frequency over weeks to months. The general physical and otological examination, routine laboratory tests, and computed tomography (CT) or magnetic resonance imaging (MRI) studies of the auditory canal and the temporal bone did not identify any cause for the hearing loss. The patients underwent an immunobiological screening, including antinuclear, antimitochondrial, anti–smooth muscle, anti–parietal cell, antithyroglobulin, antimicrosomal, and antineutrophil cytoplasmic antibodies. Two hundred fourteen randomly selected age- and sex-matched white subjects without any clinical evidence or family history of any autoimmune disease were used as normal local controls.

DNA Extraction and HLA Genotyping. For DNA extraction, genomic DNA was extracted from ethylenediaminetetraacetic acid–treated whole blood samples by means of a QIAmp column (Quiagen, Chatworth, Calif) or by the classic phenol-chloroform procedure.[9]

For PCR-SSO reverse dot blot (RDB), HLA-DRB1, -DRB3, -DRB4, -DRB5, and -DPB1 genotyping was performed by PCR-RDB hybridization assays as described by Buyse et al.[10] The Taq DNA polymerase (Amersham, Little Chalfont, England) and the INNO-LiPA reagents (Innogenetics NV, Ghent, Belgium) were used. In brief, the polymorphic second exons of the DRB1, DRB3, DRB4, and DRB5 as well as DPB1 genes were amplified by the PCR, as recommended by the manufacturer, in a Thermal Cycler (Perkin-Elmer Cetus, Norwalk, Conn). The PCR primers are shown in Table 1. The biotinylated PCR products were subsequently hybridized with a total of 31 DRB and 23 DPB1 SSOs immobilized in a linewise fashion on nitrocellulose strips. Streptavidin labeled with alkaline phosphatase was then added. A colorimetric visualization of positive hybridization signals was obtained by means of NBT/BCIP substrates. The INNO-LiPA Expert genotyping program V2.0 (Innogenetics) was used to interpret the hybridization patterns.

For PCR-RFLP, the DQB1 alleles were typed by the PCR-RFLP method as described by Sengar and Goldstein.[11] On the other hand, the samples ambiguously typed for DRB by the PCR-RDB were retested by the PCR-RFLP method.[12-14] The primers shown in Table 2 were synthesized with a Cyclone Plus DNA synthesizer (MilliGen/Biosearch, Novato, Calif) and purified as described previously.[15] The PCR products were digested with allele-specific restriction endonucleases (Amersham; and Boehringer Mannheim, Mannheim, Germany) at their respective optimal temperatures, followed by electrophoresis on 4% agarose gel (Life Technology, Paisley, Scotland) prestained with ethidium bromide. The HLA genotyping was determined by comparing the RFLP patterns with the standard patterns.

On the basis of these techniques, 88 of 106 DRB1, 4 of 4 DRB3, 2 of 3 DRB4, 4 of 5 DRB5, 22 of 23 DQB1, and 58 of 61 DPB1 alleles could be discriminated.

TABLE 2. PCR PRIMERS USED FOR HLA-DRB AND -DQB PCR-RFLP TYPING

Specificity		*Name*	*Codons*	*Sequence*	*Product Size (bp)*
DRB1	DR1	Ota-5'R1	25 to 32	GGTTGCTGGAAAGATGCATCT	206
		Ota-3'P	87 to 93	CCGCTGCACTGTGAAGCTCT	
	DR2	Ota-5'R2	7 to 13	TTCCTGTGGCAGCCTAAGAGG	261
		Ota-3'R	87 to 93	CCGCTGCACTGTGAAGCTCT	
	DR3568 I	Ole-5'05	6 to 13	GTTTCTTGGAGTACTCTACGTC	260
		Ole-3'047	86 to 92	CTGCACTGTGAAGCTCTCAC	
	DR3568 IIa	Ole-5'08	9 to 16	GTACTCTACGGGTGAGTGTT	250
		Ole-3'047	86 to 92	CTGCACTGTGAAGCTCTCAC	
	DR3568 IIb	Ole-5'08	9 to 16	GTACTCTACGGGTGAGTGTT	250
		Ole-3'048	86 to 92	CTGCACTGTGAAGCTCTCCA	
	DR3568 III	Ole-5'05	6 to 13	GTTTCTTGGAGTACTCTACGTC	260
		Ole-3'048	86 to 92	CTGCACTGTGAAGCTCTCCA	
	DR4	Ota-5'R4	6 to 13	GTTTCTTGGAGCAGGTTAAAC	263
		Ota-3'R	87 to 93	CCGCTGCACTGTGAAGCTCT	
DRB3		DRb32	8 to 13	TGGAGCTGCGTAAGTCT	257
		Ota-3'R	87 to 93	CCGCTGCACTGTGAAGCTCT	
DQB1	DQ 5, 6	SDQ-01	−1 to 8	AGGGATCCCCGCAGAGGATTTCGTG	279
		QB202	84 to 93	CACCTGCAGATCCCGCGGTACGCCACCTC	
	DQ 2, 3, 4	SDQ-01	−1 to 8	AGGGATCCCCGCAGAGGATTTCGTG	275
		QB204	83 to 92	CACCTGCAGTGCGGAGCTCCAACTGGTA	

PCR — polymerase chain reaction, RFLP — restriction fragment length polymorphism, bp — base pair.

Statistical Analysis. Single HLA allele frequency distributions were evaluated by means of the χ^2 test with Yates' correction on 2 by 2 contingency tables. Fisher's exact test was used when necessary. A level of $p < .05$ was accepted as statistically significant. Strength of association was expressed as Haldane's relative risk (RR), a modification of Wool's odds ratio.[16,17]

RESULTS

HLA-DRB1, -DRB3, -DRB4, and -DRB5 Genotyping. The distribution of the HLA-DRB alleles in 34 patients with idiopathic PSHL and 214 controls is shown in Table 3. The frequency of HLA-DRB1*0301 was significantly increased ($\chi^2 = 5.363$, $p < .05$, RR = 2.35) in patients with idiopathic PSHL as compared with controls. The frequencies of the other DRB1 alleles were not significantly different between the patient and control groups. The decrease of DRB1*0401 and DRB1*1101 in patients did not reach statistical significance. Concerning the DRB3 gene present in the DR3, DR11, DR12, DR13, and DR14 haplotypes, the DRB3*0101 allele showed a significant increase in the patient group ($\chi^2 = 6.785$, $p < .01$, RR = 2.60). Seventy-five percent of the DRB3*0101-positive patients carried the DRB1*0301 allele, and 75% of the DRB1*0301-positive patients carried the DRB3*0101 allele as well (data not shown). As an example, observed PCR-RDB and PCR-RFLP patterns are shown in Figs 1 and 2.

HLA-DQB1 Genotyping. The distribution of the HLA-DQB1 alleles defined in this study is shown in Table 4. The frequency of the DQB1*0201 allele was significantly increased ($\chi^2 = 7.947$, $p < .01$, RR = 2.38) in patients with idiopathic PSHL compared with controls. The frequency of the DQB1*0301 allele was significantly decreased ($\chi^2 = 7.712$, $p < .01$, RR = 0.29) in patients compared with controls.

HLA-DPB1 Genotyping. The DPB1 alleles assigned to patients and controls are shown in Table 5. The HLA-DPB1*0401 allele was significantly increased in patients with idiopathic PSHL ($\chi^2 = 5.294$, $p < .05$, RR = 1.88).

DISCUSSION

In 1987, Bowman and Nelson[7] reported that there was a reduced frequency of DR4 without any trends of increase of other DR specificities in patients with autoimmune sensorineural hearing loss. Another serologic typing study revealed that HLA-B8/DR3 and especially haplotypes A1/B8/DR3 were significantly increased in patients with inner ear disease, whereas B8 and DR3 per se only approached significance compared with the normal population of North America.[8] However, the present results of HLA class II genotyping by PCR-RDB and PCR-RFLP showed a significant increase of DRB1*0301 (DR3) in patients with idiopathic PSHL in the Belgian population. A

TABLE 3. FREQUENCY OF RELEVANT HLA-DR ALLELES IN PATIENTS WITH IDIOPATHIC PSHL AND IN HEALTHY CONTROLS

	Frequency (%)				
Alleles	*Patients (n = 68)*	*Controls (n = 428)*	χ^2	*p*	*RR*
DRB1*0101	8.8	8.4			
DRB1*0102	4.4	1.3			
DRB1*1501	14.7	12.8			
DRB1*1502	1.5	0.6			
DRB1*1601	1.5	2.1			
DRB1*0301	20.6	10.1	5.363	<.05	2.35
DRB1*0401	1.5	7.4			
DRB1*0402	1.5	0.8			
DRB1*0403	2.9	1.1			
DRB1*0404	2.9	4.0			
DRB1*0407	2.9	0.2			
DRB1*1101	1.5	8.4			
DRB1*1301	7.4	6.3			
DRB1*0701	11.8	11.8			
DRB3*0101	20.6	9.2	6.785	<.01	2.60
DRB3*0202	14.7	12.0			
DRB4*0101	20.6	28.4			
DRB5*0101	14.7	7.9			
DRB5*0102	1.5	0.6			
DRB5*0201	1.5	2.9			

PSHL — progressive sensorineural hearing loss, n — number of patient and control chromosomes.

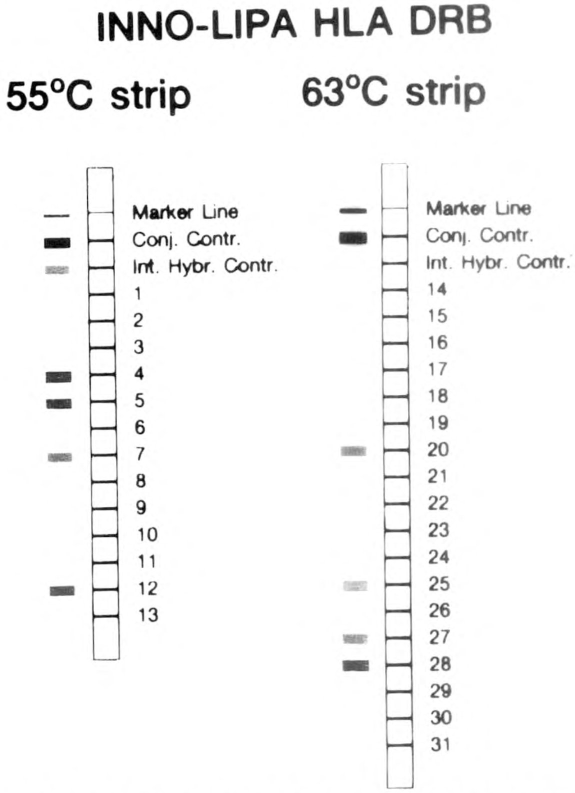

Fig 1. Hybridization pattern of polymerase chain reaction (PCR)–amplified DRB genes obtained from DNA of one individual DRB1*0101/DRB1*0301-DRB3*0101, after hybridization with 31 DRB sequence–specific oligonucleotides immobilized on nitrocellulose strips. Conj. Contr. — conjugate control, Int. Hybr. Contr. — internal hybridization control.

decrease of the DRB1*0401 allele was also observed in the patient group, but did not reach statistical significance.

Among the alleles of the other expressed DRB loci, the frequency of the DRB3*0101 allele was significantly increased. In the normal population, there is a linkage disequilibrium (preferential association of alleles corresponding to different loci) between DRB3*0101 and DRB1*0301 alleles. Indeed, DRB3 and DRB1 genes are closely located on the chromosome. In the patient series, we found a strong association between DRB3*0101 and DRB1*0301 alleles. Thus, it is difficult to identify which of these two alleles is the causative HLA factor truly responsible for the association with idiopathic PSHL. Further studies are required by increasing the sample size or investigating other ethnic groups.

The HLA-DR and -DQ genes are tightly linked, and the alleles of these loci show extremely strong linkage disequilibrium within the population, so the increase of DQB1*0201 in patients with idiopathic PSHL may be associated with the DRB1*0301 allele. We also found that the frequency of the DQB1*0301 allele was significantly decreased in patients with idiopathic PSHL. The analysis of insulin-dependent diabetes mellitus (IDDM) susceptibility associated with DQβ allelic variation has implicated that the

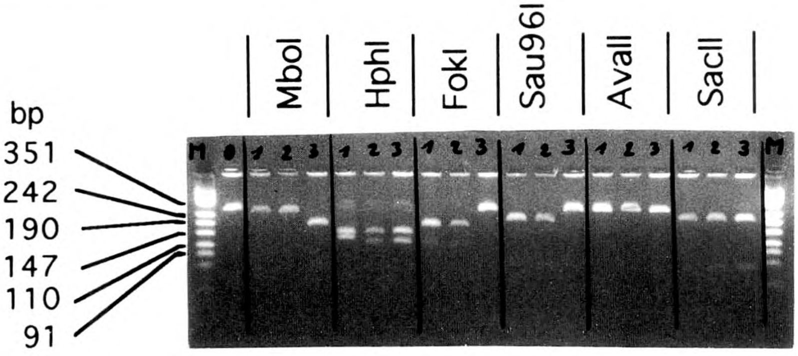

Fig 2. Cleavage patterns of polymorphic restriction fragments in PCR-amplified DRB1 genes obtained from DNAs of three individuals after digestion with six restriction enzymes. Size of amplified region of DRB1 exon 2 is 261 base pair (bp) as indicated. M — size marker, PUC18 DNA digested with Hpa II; 0 — undigested PCR product (261 bp); 1 — DRB1*1501 or 1503 sample; 2 — DRB1*1502 sample; 3 — DRB1*1601 sample.

TABLE 4. FREQUENCY OF HLA-DQB1 ALLELES IN PATIENTS WITH IDIOPATHIC PSHL AND IN HEALTHY CONTROLS

	Frequency (%)				
Alleles	*Patients (n = 68)*	*Controls (n = 428)*	χ^2	*p*	*RR*
DQB1*0501	14.7	11.3			
DQB1*0502	1.5	2.7			
DQB1*0503	2.9	1.6			
DQB1*0601	1.5	1.1			
DQB1*0602	13.2	9.8			
DQB1*0603	5.9	5.8			
DQB1*0604	5.9	3.0			
DQB1*0201	30.9	15.9	7.947	<.01	2.38
DQB1*0301	7.4	22.9	7.712	<.01	0.29
DQB1*0302	10.3	10.8			
DQB1*0402	1.5	3.2			

PSHL — progressive sensorineural hearing loss, n — number of patient and control chromosomes.

TABLE 5. FREQUENCY OF HLA-DPB1 ALLELES IN PATIENTS WITH IDIOPATHIC PSHL AND IN HEALTHY CONTROLS

	Frequency (%)				
Alleles	*Patients (n = 68)*	*Controls (n = 428)*	χ^2	*p*	*RR*
DPB1*0101	4.4	3.7			
DPB1*0201	11.8	16.4			
DPB1*0202	1.5	0.9			
DPB1*0301	5.9	13.3			
DPB1*0401	55.9	40.2	5.294	<.05	1.88
DPB1*0402	10.3	13.3			
DPB1*0501	1.5	1.2			
DPB1*1101	1.5	2.1			
DPB1*1301	1.5	2.3			
DPB1*1401	1.5	0.7			
DPB1*1901	1.5	0.5			

PSHL — progressive sensorineural hearing loss, n — number of patient and control chromosomes.

charge of DQβ polymorphic residue at position 57 plays an important role in the development or suppression of the autoimmune response.[18] Presence or absence of the charged aspartate residue at position 57 of the DQβ chain confers resistance or susceptibility to IDDM, respectively.[19,20] Whether the DQB1*0301 allele negative association with idiopathic PSHL is also related to the charged aspartate at position 57 of the DQβ chain remains to be determined.

Owing to intrinsic difficulties in its typing, low level of expression, and lack of serologic reagents, the HLA-DP region has remained an untyped candidate in patients with inner ear disease. However, several studies demonstrated that the HLA-DPB1 alleles can be associated with autoimmune disease.[21,22] By genomic typing in HLA-DPB1, we found that the frequency of DPB1*0401 was increased in patients with idiopathic PSHL. The association of the DPB1*0401 allele with idiopathic PSHL was not as strong as that observed with DRB1 and DRB3 alleles. This may be partially due to the fact that the DPB1*0401 allele predominates in the white population.

In general, DPB1 alleles are not in linkage disequilibrium with DRB and DQB alleles.[23] In the present study, we found that only 18% of the DPB1*0401 alleles were associated with the DRB1*0301 allele, and 18% of the DPB1*0401 alleles were associated with the DRB3*0101 allele as well. Moreover, all the DPB1*0401 alleles in these patients were not always encoded on the same chromosome as DRB1*0301 or DRB3*0101. On the other hand, the frequencies of haplotypes DRB1*0301/DPB1*0401 and DRB3*0101/DPB1*0401 in controls were higher than in patients with idiopathic PSHL (data not shown). Therefore, our results indicate a random association between DPB1*0401 and DRB1*0301 or DRB3*0101 in patients with idiopathic PSHL. In other words, the telomeric DPB1*0401 allele may act as an independent risk factor in the pathogenesis of inner ear disease.

The mechanism of autoimmune inner ear disease is complex and multifactorial. The expression of a particular HLA gene may not be by itself the cause of any autoimmune disease, but one of several factors that contribute to autoimmunity. Generally, idiopathic PSHL pathogenesis requires an interaction between genetic susceptibility and environmental factors. Some virus infections, such as cytomegalovirus, Lassa fever virus, and parvovirus B19, have been implicated in the pathogenesis of immune-mediated PSHL.[24-26] Under certain circumstances, the genetically predisposed individuals are at a particularly high risk. These environmental conditions possibly trigger inner ear disease in susceptible individuals and determine the phenotypic expression of idiopathic PSHL. A shared peptide sequence has been noted for the CMV IE2 protein (PLGRPD at residues 81 to 86) and residues 52 to 57 of the DRβI chain (ELGRPD).[27] The shared structures between some pathogen and particular class II molecules could serve as a cross-reactive target autoantigen for an immune response to the viral pathogen or lead to a cross-tolerance in these individuals. Knowledge of genotypes and haplotypes conferring susceptibility to idiopathic PSHL could be very useful in studying predisposition, particularly in familial cases.

In conclusion, this preliminary study shows that idiopathic PSHL is associated with HLA-DRB1*0301, DRB3*0101, DQB1*0201, and DPB1*0401 in a series of 34 consecutive patients. A negative association with HLA-DQB*0301 was also observed. The concomitant increase of both DRB1*0301 and DRB3*0101 alleles in patients with idiopathic PSHL is likely to reflect a linkage disequilibrium between these two alleles. The increase of DQB1*0201 in patients was associated with the DRB1*0301 allele. The telomeric DPB1*0401 allele may act as an independent risk factor in the pathogenesis of inner ear disease. The DQB1*0301 allele may be associated with protection from idiopathic PSHL. Nevertheless, it will be necessary to investigate further the HLA genes, and even the noncoding sequences of DNA, located within and tightly linked to the class II region, in a large population of patients.

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REFERENCES

1. Svejgaard A, Platz P, Ryder LP. HLA and disease 1982. A survey. Immunol Rev 1983;70:193-218.
2. Hughes GB, Kinney SE, Barna BP, Calabrese LH. Practical versus theoretical management of autoimmune inner ear disease. Laryngoscope 1984;94:758-67.
3. Harris JP, Sharp PA. Inner ear autoantibodies in patients with rapidly progressive sensorineural hearing loss. Laryngoscope 1990;100:516-24.
4. Joliat T, Seyer J, Bernstein J, et al. Antibodies against a 30 kilodalton cochlear protein and type II and IX collagens in the serum of patients with inner ear diseases. Ann Otol Rhinol Laryngol 1992;101:1000-6.
5. Cao MY, Gersdorff M, Deggouj N, Tomasi J-P. The localization and specificity of guinea pig inner ear antigenic epitopes. J Laryngol Otol 1995;109:20-4.
6. McCabe BF. Autoimmune sensorineural hearing loss. Ann Otol Rhinol Laryngol 1979;88:585-9.
7. Bowman CA, Nelson RA. Human leukocyte antigens in autoimmune sensorineural hearing loss. Laryngoscope 1987;97:7-9.
8. Bernstein JM, Shanahan T, Yoo TJ, Ye XJ. HLA antigens and inner ear disease. In: McCabe BF, Veldman JE, Mogi G, eds. Immunobiology in otology, rhinology and laryngology. Amsterdam, the Netherlands: Kugler Publications, 1992:3-11.
9. Wallace DM. General methods for isolating and characterizing nucleic acid. In: Berger SL, Kimmel AR, eds. Guide to molecular cloning. San Diego, Calif: Academic Press, 1987:33-48. (Abelson JN, Simon MI, eds. Methods in enzymology; vol 152.)
10. Buyse I, Decorte R, Baens M, et al. Rapid DNA typing of class II HLA antigens using the polymerase chain reaction and reverse dot blot hybridization. Tissue Antigens 1993;41:1-14.
11. Sengar DPS, Goldstein R. Comprehensive typing of DQB1 alleles by PCR-RFLP. Tissue Antigens 1994;43:242-8.
12. Sengar DPS, Goldstein R, Toye B, Hampton N. Comprehensive typing of DR52 (DRB3)-associated DRB1 and DRB3 alleles by PCR-RFLP. Tissue Antigens 1994;43:286-94.
13. Ota M, Seki T, Fukushima H, Tsuji K, Inoko H. HLA-DRB1 genotyping by modified PCR-RFLP method combined with group-specific primers. Tissue Antigens 1992;39:187-202.
14. Sengar DPS, Goldstein R. Typing of DRB3*, DRB4* and DRB5* alleles by PCR-RFLP [Abstract]. Hum Immunol 1993; 37(suppl 1):128.
15. Vaerman JL, Moureau P, Larondelle Y, Martiat P, Philippe M. Prediction of oligodeoxyribonucleotides eluting salt conditions on anion exchange column. Nucleic Acids Res 1992;20:3522.
16. Haldane JBS. The estimation and significance of the logarithm of a ratio of frequencies. Ann Hum Genet 1955;20:309-11.
17. Tiwari JL, Terasaki PI. HLA and disease associations. New York, NY: Springer-Verlag, 1985:19-27.
18. Todd JA, Bell JI, McDevitt HO. HAL-DQB contributes to susceptibility and resistance to insulin-dependent diabetes mellitus. Nature 1987;329:599-604.
19. Morel PA, Dorman JS, Todd JA, McDevitt HO, Trucco M. Aspartic acid at position 57 of the HLA-DQβ chain protects against type I diabetes: a family study. Proc Natl Acad Sci USA 1988;85:8111-5.
20. Ronningen KS, Iwe T, Halstensen TS, Spurkland A, Thorsby E. The amino acid at position 57 of the HLA-DQβ chain and susceptibility to develop insulin-dependent diabetes mellitus. Hum Immunol 1989;26:221-5.
21. Begovich AB, Bugawan TL, Nepom SB, Klitz W, Nepom GT, Erlich HA. A specific HLA-DPB allele is associated with pauciarticular juvenile rheumatoid arthritis but not adult rheumatoid arthritis. Proc Natl Acad Sci USA 1989;86:9489-93.
22. Bugawan TL, Angelini G, Larrick L, Auricchio S, Ferrara GB, Erlich HA. A combination of a particular HLA-DPβ allele and an HLA-DQ heterodimer confers susceptibility to coeliac disease. Nature 1989;339:470-3.
23. Howell WM, Evans PR, Devereux SA, Sage DA, Smith JL, Haegert DG. Absence of strong HLA-DR/DQ-DP linkage disequilibrium in the British and French Canadian caucasoid populations. Eur J Immunogen 1993;20:363-71.
24. Wilson WR, Veltri RW, Laird N, Sprinkle PM. Viral and epidemiologic studies of idiopathic sudden hearing loss. Otolaryngol Head Neck Surg 1983;91:653-8.
25. Cummins D, McCormick JB, Bennett D, et al. Acute sensorineural deafness in Lassa fever. JAMA 1990;264:2093-6.
26. Cotter CS, Singleton GT, Corman LC. Immune-mediated inner ear disease and parvovirus B19. Laryngoscope 1994;104:1235-9.
27. Oldstone MBA. Molecular mimicry and autoimmune disease. Cell 1987;50:819-20.

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CIRCULATING INTERCELLULAR ADHESION MOLECULE–1 AND ITS CELLULAR EXPRESSION IN HEAD AND NECK NON-HODGKIN'S LYMPHOMAS, INCLUDING LETHAL MIDLINE GRANULOMA

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Extranodal non-Hodgkin's lymphoma in the head and neck, especially T-cell lymphoma of the lethal midline granuloma (LMG) type, has unique clinical and histologic features differentiating it from other lymphomas. The authors measured soluble intercellular adhesion molecule–1 (ICAM-1) in sera from 12 patients with T-cell lymphoma of the LMG type and from 52 patients with other head and neck non-Hodgkin's lymphomas, by double-determinant immunoassay. The expression of ICAM-1 in lymphoma tissue was examined in 26 patients by the avidin-biotin immunoperoxidase method. The serum ICAM-1 levels were significantly higher in T-cell lymphoma of the LMG type than in other head and neck lymphomas or in healthy adult controls. Elevated levels of serum ICAM-1 were associated with increased levels of serum lactate dehydrogenase, poor prognosis, and impaired T-cell–dependent immune functions, both in T-cell lymphoma of the LMG type and in other head and neck lymphomas. When we monitored serum ICAM-1 levels in individual patients, the levels decreased in the complete remission interval compared to before treatment and went up again when the lymphoma relapsed. Although the staining intensities of ICAM-1 in lymphoma cells were not related to serum ICAM-1 levels, a markedly intense expression of ICAM-1 was found on the angiocentric or angiodestructive lymphoreticular infiltrate region in the tissues from T-cell lymphoma of the LMG type. A higher serum ICAM-1 level and its tissue expression in T-cell lymphoma of the LMG type may be one of the clues to understanding this particular lymphoma. The serum ICAM-1 level could be an efficient parameter for monitoring the clinical course of head and neck non-Hodgkin's lymphomas.

KEY WORDS — head and neck, ICAM-1, lethal midline granuloma, non-Hodgkin's lymphoma, soluble ICAM-1.

INTRODUCTION

Non-Hodgkin's lymphoma frequently arises from the head and neck region. The most common type is diffuse large B-cell lymphoma originating in Waldeyer's ring. When localized, its prognosis is favorable. However, T-cell lymphoma having clinical features of lethal midline granuloma (LMG), characterized by progressive unrelenting ulceration and necrosis of the nasal cavity and midline facial tissues, is occasionally found.[1-3] Its prognosis is markedly poor as compared to other head and neck lymphomas.[3] Histologic features of the T-cell lymphoma of the LMG type, displayed by angiocentric, polymorphous lymphoreticular infiltrates, have been called polymorphic reticulosis.[4] Phenotypically, the coexpression of the natural killer (NK) cell phenotype CD56 and pan-T antigens has been reported.[5,6] With regard to etiology, we previously showed clonotypic Epstein-Barr virus (EBV) genome and EBV-encoded proteins in 5 cases of T-cell lymphoma of the LMG type, suggesting a causal association with EBV.[7,8]

Intercellular adhesion molecule–1 (ICAM-1), a member of the immunoglobulin supergene family with a five-domain structure,[9] has been characterized as one of the natural ligands to lymphocyte function–associated antigen–1 (LFA-1) molecule expressed on all leukocytes.[9,10] The ICAM-1 is expressed on a limited number of tissues constitutively but is induced on a wide variety of normal cells, such as vascular endothelial cells, leukocytes, and some epithelial tissues.[11] Increased cellular expression of this molecule has been reported to be correlated with metastasis in melanoma,[12] but not to be associated with tumor progression in lymphoma or leukemia.[13,14] A soluble form of ICAM-1 has been found in increased levels in sera of patients with several inflammatory, immune, or malignant disorders.[15-23] Increased serum ICAM-1 level has been also associated with metastasis, tumor spread, and poor prognosis in melanoma,[20] gastrointestinal cancers,[21] and Hodgkin's disease.[22,23] Several cytokines, such as interferon-γ, tumor necrosis factor–α, and interleukin-1, have been shown to enhance the expression of this molecule.[24,25] Previous in vitro study showed that interferon-γ and tumor necrosis factor–α induce also the shedding of ICAM-1 on tumor cell lines.[20,21,26] Aside from such cytokines, EBV or EBV-encoded latent membrane protein has been reported to enhance the expression of ICAM-1.[27-29]

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In a previous report,[30] we demonstrated increased levels of serum ICAM-1 in adult T-cell leukemia, which is associated with human T-lymphotrophic virus type 1. In the present study, we demonstrate higher serum ICAM-1 levels in patients with T-cell lymphoma of the LMG type — most of these lymphomas exhibited the NK cell phenotype and were causally associated with EBV — as compared to patients with other head and neck lymphomas and healthy adult controls. Furthermore, we examine the prognostic value of serum ICAM-1 levels and its relation to cellular expression of this molecule in head and neck lymphomas.

PATIENTS AND METHODS

Patients. The study group consisted of 12 patients with T-cell lymphoma of the LMG type and 52 patients with other head and neck non-Hodgkin's lymphomas who received diagnoses and treatment in our clinic between 1983 and 1993. The primary sites of lymphomas other than LMG were Waldeyer's ring in 33 patients, nose and paranasal sinuses in 7 patients, neck lymph nodes in 7 patients, and salivary glands in 5 patients. Clinical data, partial immunohistologic reviews, and the association with EBV of 5 patients with T-cell lymphoma of the LMG type have been reported elsewhere.[7,8,31] The follow-up period of living patients was from 9 to 101 months, with 46 months as the median value.

Clinicohistologic and Immunologic Evaluations and Treatments. Pertinent clinical information and follow-up data were obtained from hospital charts for all cases. The clinical stage according to the Ann Arbor system[32] was assessed by physical examination, gallium 67 scintiscan, computed tomography scan, echogram, radiographic and fiberscopic examination of the gastrointestinal tract, and bone marrow aspiration. The histologic type was classified according to the Working Formulation System of the National Cancer Institute.[33] The phenotype of lymphoma cells was studied on formalin-fixed, paraffin-embedded sections by the avidin-biotin immunoperoxidase method, as described previously.[34] Monoclonal antibody, UCHL1/CD45RO (Dakopatts, Copenhagen, Denmark) or MT1 (Bio-Science, Emmenbrucke, Switzerland), was used for pan-T marker, and L26 (Dakopatts) was employed for pan-B antigen. In 7 cases of T-cell lymphoma of the LMG type and 4 cases of other T-cell lymphomas, the NK-cell phenotype CD56 was examined on fresh-frozen sections by means of NKH-1 monoclonal antibody (Coulter Clone, Hialeah, Fla). The phenotype was defined as over 70% positive-staining lymphoma cells counted in the area involving atypical mononuclear cells. The T-cell–dependent immune function before treatments was assessed by delayed-type hypersensitivity reactions or by the CD4/CD8 ratio of peripheral blood lymphocytes (PBLs). The delayed-type hypersensitivity was elicited in 24 patients by skin reactions to purified protein derivative prepared from *Mycobacterium tuberculosis*, *Candida albicans* extracts, or phytohemagglutinin after 48 hours of injections. A positive response was defined as erythema of at least 5 mm in long diameter on the purified protein derivative or *Candida* test, and by erythema of at least 15 mm on the phytohemagglutinin test. If the responses were heterogeneous among antigens, the corresponding result between two skin tests was scored. The CD4/CD8 ratio of PBLs was measured in 44 patients. The percentages of CD4- and CD8-positive cells were analyzed by flow cytometry (FACScan; Becton-Dickinson, Mountain View, Calif) after fluorescein isothiocyanate immunofluorescence staining using Leu-4 and Leu-8 monoclonal antibodies (Becton-Dickinson). The treatment plan was as follows. Patients in stage I were treated only with cobalt 60 irradiation (40 to 45 Gy). Patients in stage II were given combined treatment with radiotherapy and chemotherapy with the VEPA[35] (vincristine, cyclophosphamide, prednisolone, doxorubicin) or the MACOP-P (vincristine, pirarubicin, cyclophosphamide, methotrexate, prednisolone, peplomycin) regimen, in which peplomycin is used instead of bleomycin, modified from the original MACOP-B regimen.[36] Patients with T-cell lymphoma of the LMG type were treated with combined radiotherapy and chemotherapy of the MACOP-P regimen, even though in stage I. Patients in stage III or IV were treated with the MACOP-P regimen with over 3 units.

Soluble ICAM-1 Assay. One serum sample was obtained from each of 64 patients before treatment. From 14 of the 64 patients, 10 samples were available at the time of complete remission confirmed after treatment and 5 sera were obtained at the time of tumor relapse discovered after complete remission. Control sera were obtained from 25 healthy adults. All samples were stored at –80°C until use. Soluble ICAM-1 in sera was measured by a double-determinant immunoassay using the FAST system (Becton-Dickinson) and two monoclonal antibodies, HA58 and CL207, as described previously.[20,21] Briefly, the beads, attached to the lip of 96-well microtiter plates, were first incubated with a monoclonal antibody, CL207, recognizing a certain part of the ICAM-1 antigen, and then blocked by 3% bovine serum albumin. The serum samples, diluted with 1:200 in phosphate-buffered saline, were then incubated with the beads. After being washed with phosphate-buffered saline containing 0.05% Tween 20, the beads were incubated with a biotinylated monoclonal antibody,

TABLE 1. CLINICOPATHOLOGIC FEATURES OF T-CELL LYMPHOMA OF LETHAL MIDLINE GRANULOMA TYPE AND OTHER HEAD AND NECK NON-HODGKIN'S LYMPHOMAS

	T-Cell Lymphoma of LMG Type (N = 12)	*Head and Neck Lymphomas Other Than LMG (N = 52)*	*p**
Age (y)			
Mean ± SD	45 ± 15	56 ± 15	.03
Range	27-71	15-74	
Male (No. of cases)	7 (58%)	27 (52%)	NS
Histologic type (WF; No. of cases)			
Low and intermediate grades	8 (67%)	44 (85%)	
High grade	4 (33%)	8 (15%)	NS
Cell type (No. of cases)			
B-cell	0 (0%)	45 (87%)	
T-cell	12 (100%)	7 (13%)	.0001
Clinical stage (No. of cases)			
I or II	11 (92%)	45 (85%)	
III or IV	1 (8%)	7 (15%)	NS
Symptoms (No. of cases)			
A	3 (25%)	47 (90%)	
B	9 (75%)	5 (10%)	.0001
PBL counts (mean ± SD)	1,285 ± 655	1,912 ± 738	.025
Serum albumin levels (mean ± SD g/dL)	3.1 ± 0.5	3.7 ± 0.3	.0001
Serum LDH levels (mean ± SD IU/L)	406 ± 192	361 ± 180	NS
Prognosis (No. of cases)			
Alive in complete remission	3 (25%)	36 (69%)	
Dead because of incomplete remission or lymphoma relapse	9 (75%)	16 (31%)	.0123
Overall 3-year survival rate†	12%	73%	<.05

LMG — lethal midline granuloma, WF — Working Formulation, PBL — peripheral blood lymphocyte, LDH — lactate dehydrogenase, NS — not significant.

*Determined by appropriate procedures, including χ^2 test, Mann-Whitney U test, or Fisher's exact test.

†Kaplan-Meier method, with statistical significance determined by Cox-Mantel test, is used.

HA58. Avidin-labeled peroxidase (Vector Laboratories, Burlingame, Calif) was diluted 1/1,000 in 0.05 mol/L phosphate-buffered saline with 0.5 mol/L sodium chloride, pH 8.0, and incubated with the beads. The degree of substrate reaction was determined with *o*-phenylenediamine at 492 nm in a micro-ELISA Autoreader MR 580 (Dynatech, Cambridge, Mass). Results were expressed as units per milliliter (1 unit/mL corresponds to 2 ng/mL of purified antigen) calculated from the titration curve of purified ICAM-1 antigen.

Cellular ICAM-1 Staining in Tissue. Twenty-six patients, from whom fresh-frozen biopsy samples were available, were examined for expression of ICAM-1 in lymphoma tissues by the avidin-biotin immunoperoxidase method[34] using monoclonal antibody HA58. The staining intensity of the lymphoma cells was scored semiquantitatively on a scale of 0 to 3 (0 being negative, 1+ weak, 2+ moderate, and 3+ strong). Frozen palatine tonsil sections were used as controls and were run in parallel in each batch of experiments. The tonsillar epithelial cells were scored 3+, the germinal center cells were scored as 2+, and the mantle zone cells were negative for ICAM-1. If staining of lymphoma cells was heterogeneous in the individual lymphoma tissue, the predominant staining intensity was scored.

Statistical Analysis. Comparisons between two groups were tested by the χ^2 test or appropriate nonparametric test procedures, including the Mann-Whitney U test, Wilcoxon signed-rank test, and Fisher's exact test. Correlation between two groups was determined by Spearman's regression coefficient. All statistical tests were based on a level of significance of $p < .05$.

RESULTS

Patient Characteristics. Clinicopathologic findings of 12 patients with T-cell lymphoma of the LMG type and of 52 patients with other head and neck non-Hodgkin's lymphomas are summarized in Table 1. The histologic type of T-cell lymphoma of the LMG type was classified as the diffuse mixed small and large cell type or the large cell, immunoblastic polymorphous type, according to the Working Formulation classification. In lymphomas other than LMGs,

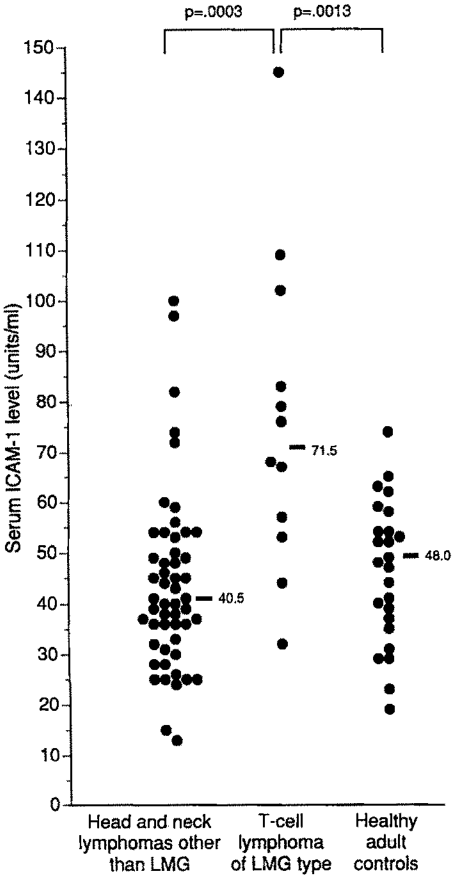

Fig 1. Serum intercellular adhesion molecule–1 (ICAM-1) levels of patients with non-Hodgkin's lymphomas of head and neck. Median values are given by thick bars. P values were determined by Mann-Whitney U test. LMG — lethal midline granuloma.

diffuse large cell B-cell lymphomas were most common (35 of 52; 67%) and T-cell lymphomas were minor (7 of 52; 13%). The NK-cell phenotype CD56 was expressed in all 7 cases of T-cell lymphoma of the LMG type tested, but was never found in all 4 cases of other T-cell lymphomas tested. In all lymphoma cases, patients with early stages (stage I or II) were more common (56 of 64; 88%) than patients with advanced stages (stage III or IV; 8 of 64; 13%). It was noteworthy that systemic symptoms, decreased PBL counts, and lower levels of serum albumin were seen more frequently in T-cell lymphoma of the LMG type than in other head and neck lymphomas. With regard to T-cell–dependent immune function before treatment, the negative response in delayed-type hypersensitivity skin reactions was shown in 7 of 8 (88%) patients with T-cell lymphomas of the LMG type tested, while it was seen in only 5 of 11 (31%) patients with other head and neck lymphomas tested ($p < .05$). Nine of 12 (75%) patients with T-cell lymphomas of the LMG type died of incomplete remission or lymphoma relapse, while 16 of 52 (31%) patients with other lymphomas died ($p = .0123$). The overall 3-year survival of patients with T-cell lymphomas of the LMG type, 12%, was significantly worse than that of patients with other head and neck lymphomas (73%, $p < .05$).

Serum ICAM-1 Levels in Head and Neck Lymphomas. The average level of serum ICAM-1 in patients with head and neck lymphomas other than LMG (mean ± SD 44 ± 18 units/mL, median 40.5 units/mL, range 13 to 100 units/mL) was not different from that in healthy adult controls (mean 46 ± 14 units/mL, median 48, range 19 to 74). There was no difference in the levels, also, among initial sites of the lymphomas. However, as shown in Fig 1, patients with T-cell lymphoma of the LMG type showed significantly higher levels of serum ICAM-1 (mean 76 ± 31 units/mL, median 71.5, range 32 to 145) than healthy adult controls ($p = .0025$) or patients with other head and neck lymphomas ($p = .0003$). When the normal value of serum ICAM-1 was set as under the mean ± 2 SD of the 25 healthy adult sera, under 75 units/mL, the incidence of positivity of serum ICAM-1 in T-cell lymphoma of the LMG type was significantly higher than that in other head and neck lymphomas (6 of 12 or 50% and 3 of 52 or 6%, respectively; $p < .001$).

Serum ICAM-1 Levels According to Clinicopathologic and Immunologic Findings. Relationships between serum ICAM-1 levels and various parameters were analyzed both in T-cell lymphoma of the LMG type and in other lymphomas, because their clinicopathologic findings and the serum ICAM-1 levels were markedly different, as shown in Table 1. With regard to lymphoma cell type, there was no difference in serum ICAM-1 levels between B-cell lymphomas (mean 43 ± 17 units/mL, median 41, range 13 to 97) and T-cell lymphomas other than LMG (mean 48 ± 25 units/mL, median 38, range 28 to 100); however, T-cell lymphoma of the LMG type had a significantly increased serum ICAM-1 level as compared to B-cell lymphoma ($p = .003$) and to other T-cell lymphomas ($p = .031$). The serum ICAM-1 levels were not related to histologic grade, clinical stage, patient's age, PBL counts, or serum albumin levels, in either group of lymphomas (Tables 2 and 3). Although patients with systemic symptoms (B symptoms) tended to have high serum ICAM-1 levels as compared to patients without systemic symptoms (A symptoms) in lymphomas other than LMG ($p = .056$), the difference was not seen in T-cell lymphoma of the LMG type (Table 2). However, serum lactate dehydrogenase levels were related positively to serum ICAM-1 levels in T-cell lymphoma of the LMG type as well as in other head and neck lymphomas ($r = .818$ and $r = .365$, respectively; $p < .01$ each; Table 3). Higher serum ICAM-1 levels were associated also with impaired T-

TABLE 2. SERUM ICAM-1 LEVELS OF PATIENTS WITH T-CELL LYMPHOMA OF LETHAL MIDLINE GRANULOMA TYPE AND OTHER HEAD AND NECK NON-HODGKIN'S LYMPHOMAS, ACCORDING TO CLINICAL, HISTOLOGIC, AND IMMUNOLOGIC FINDINGS

	T-Cell Lymphoma of LMG Type (n = 12)		*Lymphomas Other Than LMG (n = 52)*	
	*Serum ICAM-1 Levels**	*p†*	*Serum ICAM-1 Levels**	*p†*
Histologic types (WF)				
Low or intermediate grades	71.5 (n = 8; 32-145)		40 (n = 44; 13-97)	
High grade	75 (n = 4; 57-109)	.001	45 (n = 8; 25-100)	NS
Cell type				
B-cell			41 (n = 45; 13-97)	
T-cell			38 (n = 7; 28-100)	NS
Clinical stage				
I or II	75 (n = 11; 32-145)		40 (n = 45; 13-97)	
III or IV	57 (n = 1)		49 (n = 7; 25-100)	NS
Symptoms				
A	44 (n = 3; 32-83)		40 (n = 47; 13-97)	
B	75 (n = 9; 53-145)	NS	54 (n = 5; 33-100)	.056
Delayed-type hypersensitivity skin reactions				
Positive response	51 (n = 1)		28 (n = 11; 24-59)	
Negative response	83 (n = 7; 57-145)		56 (n = 5; 39-100)	.019
CD4/CD8 ratio of PBLs				
≥1.2	60 (n = 6; 32-75)		38 (n = 31; 13-82)	
<1.2	109 (n = 3; 102-145)	.02	53.5 (n = 4; 41-60)	.048
Prognosis				
Alive in complete remission	44 (n = 3; 32-68)		38.5 (n = 36; 13-97)	
Dead because of incomplete remission or lymphoma relapse	79 (n = 9; 53-145)	.052	47.5 (n = 16; 30-100)	.018

LMG — lethal midline granuloma, WF — Working Formulation, PBLs — peripheral blood lymphocytes, NS — not significant.

*Results are expressed as median values (number; range) in units per milliliter. One unit corresponds to 2 ng of purified antigen.

†Determined by Mann-Whitney U test.

cell–dependent immune functions, both in T-cell lymphoma of the LMG type and in other head and neck lymphomas, as shown in Table 2. In lymphomas other than LMG, patients with a negative response in delayed-type hypersensitivity skin reactions had significantly higher serum ICAM-1 levels (mean 64 ± 23 units/mL, median 56, range 39 to 100) than patients with a positive response (mean 37 ± 14 units/mL, median 28, range 24 to 59; p = .019). The serum ICAM-1 level of patients with a low CD4/CD8 ratio (<1.2) of PBLs was significantly higher than that of patients with a normal CD4/CD8 ratio (≥1.2) in T-cell lymphoma of the LMG type (mean 119 ± 23 units/mL, median 109, range 102 to 145, and mean 57 ± 16 units/mL, median 60, range 32 to 75, respectively; p = .02), as well as in other head and neck lymphomas (mean 52 ± 8 units/mL, median 53.5, range 41 to 60, and mean 41 ± 15 units/mL, median 38, range 13 to 82, respectively; p = .048; Table 2).

TABLE 3. CORRELATIONS BETWEEN SERUM ICAM-1 LEVELS AND CLINICAL PARAMETERS IN T-CELL LYMPHOMA OF LETHAL MIDLINE GRANULOMA TYPE AND OTHER HEAD AND NECK NON-HODGKIN'S LYMPHOMAS

	T-Cell Lymphoma of LMG Type	*Lymphomas Other Than LMG*
Serum ICAM-1 levels versus		
Age	.035	.274
PBL counts	–.3	.178
Serum albumin levels	–.263	–.12
Serum LDH levels	.818*	.365*

Data are expressed as r values determined by Spearman's rank correlation.

LMG — lethal midline granuloma, PBL — peripheral blood lymphocyte, LDH — lactate dehydrogenase.

*p < .01.

Serum ICAM-1 Levels According to Clinical Course. As shown in Table 2, the serum ICAM-1 levels before treatment in patients who died of incomplete remission or relapse after complete remission tended to be high as compared with those in patients who were alive after complete remission of T-cell lymphoma of the LMG type (mean ± SD 86 ± 29 units/mL, median 79, range 53 to 145, and mean

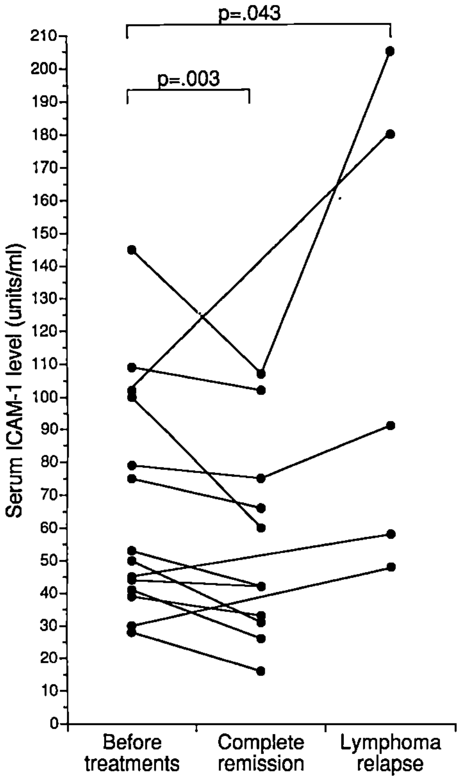

Fig 2. Changes of serum ICAM-1 levels during clinical course in each individual patient. Notice that all patients show unchanged or decreased levels (54 ± 30 units/mL) in complete remission interval, ie, significantly lower than before treatment (67 ± 35 units/mL, p = .003). Likewise, patients with tumor relapse after complete remission exhibit significantly elevated levels (116 ± 72 units/mL) as compared to levels before treatment (p = .043). P values were determined by Wilcoxon signed-rank test.

48 ± 18 units/mL, median 44, range 32 to 68, respectively; p = .052). Furthermore, in head and neck lymphomas other than LMG, patients with a poor prognosis showed significantly higher serum ICAM-1 levels before treatment (mean 52 ± 19 units/mL, median 47.5, range 30 to 100) than patients with a favorable prognosis (mean 40 ± 16 units/mL, median 38.5, range 13 to 97; p = .018).

Follow-up data of serum ICAM-1 levels were available in 14 patients with head and neck lymphomas, including T-cell lymphoma of the LMG type, as shown in Fig 2. Comparing serum ICAM-1 levels before and after treatment in each individual patient, the levels at the time complete remission was confirmed (54 ± 30 units/mL) were significantly lower than the levels before treatment (67 ± 35 units/mL, p = .003). On the other hand, the serum ICAM-1 levels at the time of lymphoma relapse discovered after complete remission were significantly elevated (116 ± 72 units/mL) compared to the levels before treatment (p = .043).

Expression of ICAM-1 in Lymphoma Tissues. Tissue expression of ICAM-1 was investigated in 10 cases of T-cell lymphoma of the LMG type, in 14 cases of diffuse B-cell lymphoma, and in 2 cases of T-cell lymphoma other than LMG. In all cases, ICAM-1 was expressed in lymphoma cells, at a weak (1+) or moderate (2+) staining intensity. Twelve cases were scored as 2+, in which lymphoma cells with a moderate staining intensity were scattered in the predominantly weakly positive tumor cell infiltrates. The remaining 14 cases were 1+, because of weak staining of most lymphoma cells. The staining intensity of ICAM-1 in lymphoma cells was not related to histologic grade, clinical stage, phenotype, patient's age, PBL count, serum albumin level, serum lactate dehydrogenase level, or T-cell–dependent immune function. The average serum ICAM-1 level of patients with 1+ staining intensity was not different from that of patients with 2+ staining (mean ± SD 60 ± 36 units/mL, median 51.5, range 13 to 145, and mean 54 ± 19 units/mL, median 49.5, range 25 to 97, respectively). In comparing T-cell lymphomas of the LMG type and other head and neck lymphomas, the staining intensities of ICAM-1 in lymphoma cells were not different. However, in the tissues from T-cell lymphomas of the LMG type, strong ICAM-1 expression (staining intensity of +3) was seen in the angiocentric or angiodestructive lymphoreticular infiltrate region and in a large number of infiltrating macrophages, which staining intensities appeared to be high as compared to lymphoma tissues other than LMG (Fig 3).

DISCUSSION

The data presented in this study demonstrated significantly increased serum ICAM-1 levels in patients with T-cell lymphoma of the LMG type as compared to patients with other head and neck lymphomas and healthy adult controls. The histologic features of T-cell lymphoma of the LMG type are diffuse infiltrates of pleomorphic large lymphoid cells and atypical small lymphoid cells, admixing with a large number of inflammatory cells such as granulocytes, macrophages, and plasma cells and with extensive ischemic necrosis. Angiocentric or angiodestructive lymphoreticular infiltrates, displayed by vascular proliferation and reticular fiber development that show a meshwork of encircling groups of and pockets of four or more loosely populated lymphoid cells, are also prominent.[2,4,7] In this study, although the ICAM-1 staining intensities of lymphoma cells in T-cell lymphoma of the LMG type appeared to be equal to those of other head and neck lymphomas at a weak or moderate level, a markedly intense expression of ICAM-1 was found in the angiocentric or angiodestructive lymphoreticular infiltrate region and in a large number of infiltrating macrophages of the tissues from T-cell lymphoma of

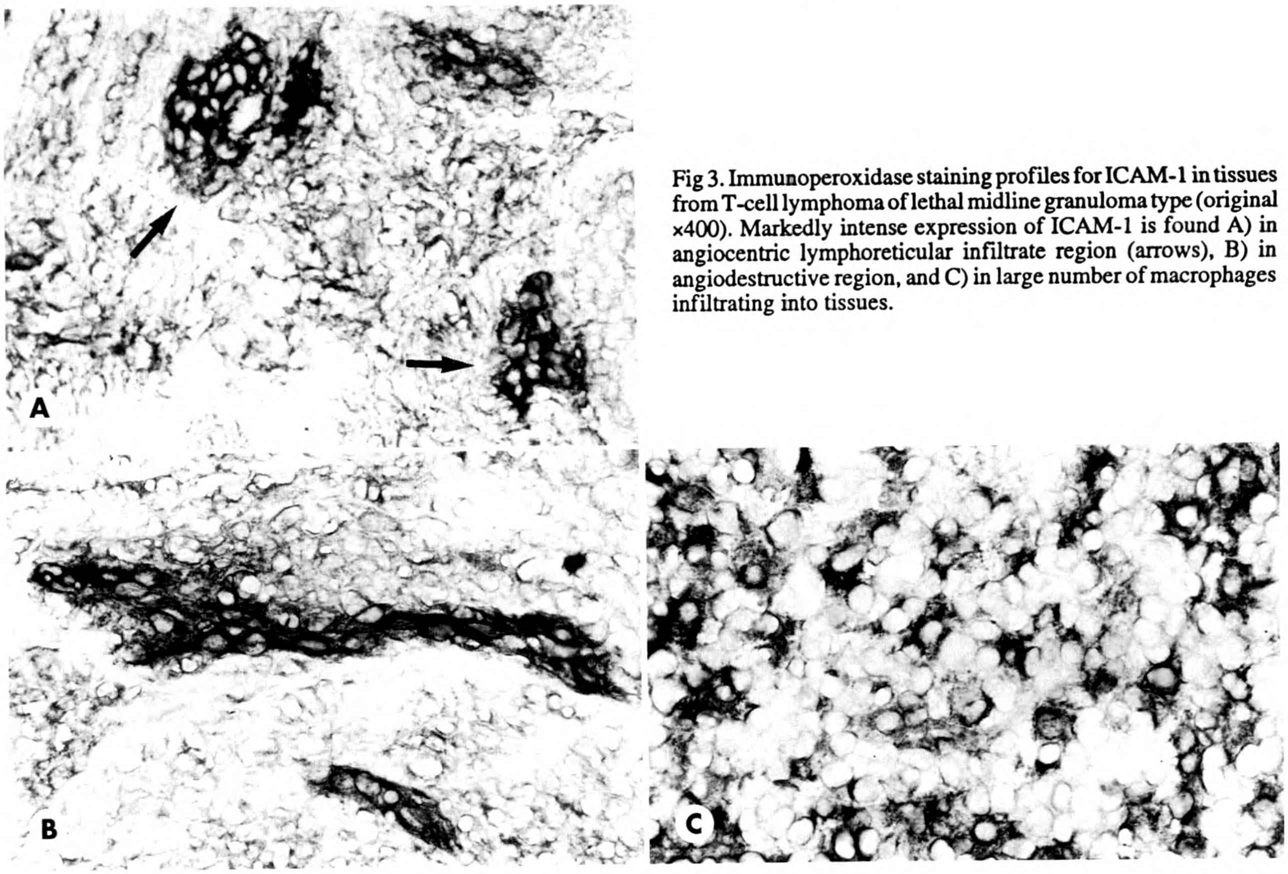

Fig 3. Immunoperoxidase staining profiles for ICAM-1 in tissues from T-cell lymphoma of lethal midline granuloma type (original ×400). Markedly intense expression of ICAM-1 is found A) in angiocentric lymphoreticular infiltrate region (arrows), B) in angiodestructive region, and C) in large number of macrophages infiltrating into tissues.

the LMG type. It has been reported that lymphoma cells of LMG expressed the NK cell phenotype CD56, as well as pan-T antigens.[5,6] Indeed, all 7 cases tested showed the coexpression of CD56 and pan-T antigens. Previous in vitro studies showed that several cytokines such as interferon-γ, tumor necrosis factor–α, and interleukin-1 enhance cellular expression, as well as shedding of ICAM-1 on tumor cells.[20,21,24-26] Therefore, it is possible that large quantities of soluble ICAM-1 may be shed into serum from damaged vascular endothelial cells and/or lymphoma cells in the angiocentric or angiodestructive lymphoreticular region, or from infiltrating macrophages, induced by cytokines released from neoplastic T-cells with the NK cell phenotype, resulting in an elevated serum ICAM-1 level in T-cell lymphoma of the LMG type. Alternatively, on the basis of a causal association of this particular lymphoma with EBV, another interesting possibility could be implied. Previously, we showed clonotypic EBV genomes and EBV-encoded protein in 5 patients (4 cases were included in this study) with T-cell lymphoma of LMG.[7,8] In 12 cases of T-cell lymphoma of the LMG type enrolled in this study, EBV genetic DNA and EBV-encoded latent membrane protein were detected in all 10 and 7 cases tested, respectively (unpublished data). Previous in vitro reports showed that EBV or latent membrane protein could enhance the expression of ICAM-1 on a nasopharyngeal carcinoma cell line, a murine B-cell line, and several EBV-positive or -negative cell lines.[27-29] It is reasonable to speculate that the existence of the virus may provide a significant contribution to the expression and/or shedding of ICAM-1 in this particular lymphoma. In another report,[30] we demonstrated increased serum ICAM-1 levels in adult T-cell leukemia in which human T-lymphotrophic virus type 1 was integrated. Tumor cells have been reported as another candidate for the source of soluble ICAM-1. In this study, although ICAM-1 expression was seen in lymphoma cells in all cases tested, the staining intensities were not related to the serum ICAM-1 levels. Further in vitro and in situ studies will be needed to clarify the source of shedding of ICAM-1.

The functional role of soluble ICAM-1 is not clear yet. The interaction of ICAM-1 and LFA-1 is crucial in non–major histocompatibility complex–restricted cell interactions.[25,37] Serum ICAM-1 retains the ability to bind specifically to LFA-1, and thus could block LFA sites on circulating T-cells. This would ultimately lead to an impairment of the adhesiveness to cellular ICAM-1 and, as a consequence, T-cell–dependent immune functions, including host antitumor immunity, would be blunted. In fact, in this study, increased serum ICAM-1 levels were closely associated with impaired T-cell–dependent immune functions assessed by delayed-type hypersensitivity skin reactions or the CD4/CD8 ratio of PBLs, both in T-cell lymphoma of the LMG type and in other head and neck lymphomas. The shedding of ICAM-1 could also block the attachment of killer T, NK, and/or lymphokine-activated killer cells to tumor cells, leading tumor cells to escape the immunosurveillance system of the host. In such a way, frequent widespread dissemination or relapse into distant sites may occur in T-cell lymphoma of the LMG type having a large amount of soluble ICAM-1. The clinical observation that serum ICAM-1 became higher as the lymphoma relapsed in some cases could support this speculation.

Serum ICAM-1 has been proposed as a new clinical marker in several malignancies such as melanoma, gastrointestinal cancers, and Hodgkin's disease, because the higher levels were correlated with metastasis, tumor spread, and poor prognosis.[20-23,38] In this study, elevated serum levels of soluble ICAM-1 were associated with increased levels of serum lactate dehydrogenase, which is one of the lymphoma progression markers, as well as with a poor prognosis both in T-cell lymphoma of the LMG type and in other head and neck lymphomas. When serum ICAM-1 levels were monitored in individual patients, the levels decreased in the complete remission interval compared to before treatment and went up again when the lymphoma relapsed. Therefore, it is suggested that serum ICAM-1 level could be a useful parameter for monitoring the clinical course of individual patients with head and neck non-Hodgkin's lymphomas, including T-cell lymphoma of the LMG type.

REFERENCES

1. Ishii Y, Yamanaka N, Ogawa K, et al. Nasal T-cell lymphomas as a type of so-called "lethal midline granuloma." Cancer 1982;50:2336-44.

2. Yamanaka N, Kataura A, Sambe S, Minase T, Ishii Y. Midfacial T cell lymphoma: characterization by monoclonal antibodies. Ann Otol Rhinol Laryngol 1985;94:207-11.

3. Yamanaka N, Harabuchi Y, Sambe S, et al. Non-Hodgkin's lymphoma of Waldeyer's ring and nasal cavity. Cancer 1985;56: 768-76.

4. Eichel BS, Harrison EG, Devine KD, Scanlon PW, Brown HA. Primary lymphoma of the nose including a relationship to lethal midline granuloma. Am J Surg 1966;112:597-605.

5. Ng CS, Chan JK, Lo ST. Expression of natural killer cell markers in non-Hodgkin's lymphomas. Hum Pathol 1987;18: 1257-62.

6. Ho FCS, Choy D, Loke SL, et al. Polymorphic reticulosis and conventional lymphomas of the nose and upper aerodigestive tracts: a clinicopathologic study of 70 cases, and immunophenotypic studies of 16 cases. Hum Pathol 1990;21:1041-50.

7. Harabuchi Y, Yamanaka N, Kataura A, et al. Epstein-Barr virus in nasal T-cell lymphomas in patients with lethal midline granuloma. Lancet 1990;335:128-30.

8. Minarovits J, Hu L-F, Imai S, et al. Clonality, expression and methylation patterns of the Epstein-Barr virus genomes in lethal midline granulomas classified as peripheral angiocentric T cell lymphomas. J Gen Virol 1994;75:77-84.

9. Dustin ML, Stauton DL, Springer TA. Supergene families meet in the immune system. Immunol Today 1988;9:213-5.

10. Marlin SD, Springer TA. Purified intercellular adhesion molecule 1 (ICAM-1) is a ligand for lymphocyte-associated antigen–1 (LFA-1). Cell 1987;51:813-9.

11. Smith MEF, Thomas JA. Cellular expression of lymphocyte function associated antigens and the intercellular adhesion molecule–1 in normal tissue. J Clin Pathol 1990;43:893-900.

12. Johnson JP, Stade BG, Holzmann B, Schwäble W, Riethmüller G. *De novo* expression of intercellular-adhesion molecule 1 in melanoma correlates with increased risk of metastasis. Proc Natl Acad Sci USA 1989;86:641-4.

13. Horst E, Meijer CJ, Radaszkiewicz T, Ossekoppele GJ, Van KJ, Pals ST. Adhesion molecules in the prognosis of diffuse large-cell lymphoma: expression of a lymphocyte homing receptor (CD44), LFA-1 (CD11a/18), and ICAM-1 (CD54). Leukemia 1990;4:595-9.

14. Archimbaud E, Thomas X, Campos L, Magaud J-P, Dore JF, Fiere D. Expression of surface adhesion molecules CD54 (ICAM-1) and CD58 (LFA-3) in adult acute leukemia: relationship with initial characteristics and prognosis. Leukemia 1992; 6:265-71.

15. Rothlein R, Mainolfi EA, Czajkowski M, Marlin SD. A form of circulating ICAM-1 in human serum. J Immunol 1991; 147:3788-93.

16. Fukukawa S, Imai K, Matsubara T, et al. Increased levels of circulating intercellular adhesion molecule 1 in Kawasaki disease. Arthritis Rheum 1992;35:672-7.

17. Shijubo N, Imai K, Aoki S, et al. Circulating intercellular adhesion molecule–1 (ICAM-1) antigen in sera of patients with idiopathic pulmonary fibrosis. Clin Exp Immunol 1992;89:58-62.

18. Aoki S, Imai K, Yachi A. Soluble intercellular adhesion molecule–1 (ICAM-1) antigen in patients with rheumatoid arthritis. Scand J Immunol 1993;38:485-90.

19. Banks RE, Gearing AJ, Hemingway IK, Norfolk DR, Perren TJ, Selby PJ. Circulating intercellular adhesion molecule–1 (ICAM-1), E-selectin and vascular cell adhesion molecule–1 (VCAM-1) in human malignancies. Br J Cancer 1993; 68:122-4.

20. Kageshita T, Yoshii A, Kimura T, Ono T. Analysis of expression and soluble form of intercellular adhesion molecule–1 in malignant melanoma. J Dermatol 1992;19:836-40.

21. Tsujisaki M, Imai K, Hirata H, et al. Detection of circulating intercellular adhesion molecule–1 antigen in malignant diseases. Clin Exp Immunol 1991;85:3-8.

22. Pizzolo G, Vinante F, Nadali G, et al. ICAM-1 tissue overexpression associated with increased serum levels of its soluble form in Hodgkin's disease. Br J Haematol 1993;84:161-2.

23. Pui CH, Luo X, Evans W, et al. Serum intercellular

adhesion molecule–1 in childhood malignancy. Blood 1993;82: 895-8.

24. Rothlein R, Czajkowski M, O'Neill MM, Marlin SD, Mainolfi EA, Merluzzi VJ. Induction of intercellular adhesion molecule 1 on primary and continuous cell lines by pro-inflammatory cytokines. Regulation by pharmacologic agents and neutralizing antibodies. J Immunol 1988;141:1665-9.

25. Springer TA. Adhesion receptors of the immune system. Nature 1990;346:425-34.

26. Becker JC, Dummer R, Hartmann AA, Burg G, Schmidt RE. Shedding of ICAM-1 from human melanoma cell lines induced by IFN-gamma and tumor necrosis factor–alpha. Functional consequences on cell-mediated cytotoxicity. J Immunol 1991;147:4398-401.

27. Wang F, Gregory C, Sample C, et al. Epstein-Barr virus latent membrane protein (LMP1) and nuclear proteins 2 and 3C are effectors of phenotypic changes in B lymphocytes: EBNA-2 and LMP1 cooperatively induce CD23. J Virol 1990;64:2309-18.

28. Salcedo R, Fuerstenberg SM, Patarroyo M, Winberg G. The Epstein-Barr virus BNLF-1 membrane protein LMP1 induces homotypic adhesion mediated by Cd11a/CD18 in a murine B-cell line, mimicking the action of phorbol ester. J Virol 1991; 65:5558-63.

29. Busson P, Zhang Q, Guillon JM, et al. Elevated expression of ICAM1 (CD54) and minimal expression of LFA3 (CD58) in Epstein-Barr–virus–positive nasopharyngeal carcinoma cells. Int J Cancer 1992;50:863-7.

30. Imai K, Nakano T, Motoya S, et al. Increased circulating intercellular adhesion molecule–1 in adult T cell leukemia patients. Int J Hematol 1992;56:239-40.

31. Harabuchi Y, Kataura A, Kobayashi K, et al. Lethal midline granuloma (peripheral T-cell lymphoma) after lymphomatoid papulosis. Cancer 1992;70:835-9.

32. Carbone PP, Kaplan HS, Musshoff K, Smithers DW, Tubiana M. Report of the Committee on Hodgkin's Disease Staging Classification. Cancer Res 1971;31:1860-1.

33. The Non-Hodgkin's Lymphoma Pathologic Classification Project. National Cancer Institute sponsored study of classifications of non-Hodgkin's lymphomas. Summary and description of a Working Formulation for clinical usage. Cancer 1982;49:2112-35.

34. Harabuchi Y, Yamanaka N, Kataura A. Identification of lymphocyte subsets and natural killer cells in head and neck cancers. An immunohistological study using monoclonal antibodies. Arch Otorhinolaryngol 1985;242:89-97.

35. Lymphoma Study Group. Combination chemotherapy and vincristine, cyclophosphamide (Endoxan), prednisolone, and Adriamycin (VEPA) in advanced adult non-Hodgkin's lymphoid malignancies: relation between T-cell or non–T-cell phenotype and response. Jpn J Clin Oncol 1979(suppl 9):397-406.

36. Klimo P, Connors JM. MACOP-B chemotherapy for the treatment of diffuse large-cell lymphoma. Ann Intern Med 1985; 102:596-602.

37. Wawryk SO, Novotny JR, Wicks IP, et al. The role of the LFA-1/ICAM-1 interaction in human leukocyte homing and adhesion. Immunol Rev 1989;108:135-61.

38. Harning R, Mainolfi E, Bystryn J-C, Henn M, Merluzzi VJ, Rothlein R. Serum levels of circulating intercellular adhesion molecule 1 in human malignant melanoma. Cancer Res 1991; 51:5003-5.

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SECRETORY OTITIS MEDIA IN ADULTS: I. THE ROLE OF MASTOID PNEUMATIZATION AS A RISK FACTOR

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TEL-AVIV, ISRAEL

We analyzed clinically 102 ears with secretory otitis media (SOM) belonging to 72 adult patients who during their adult life had not suffered previously from ear disease. As in children, most of the cases (63%) could be traced directly to an upper respiratory tract infection. The most striking finding was the preponderance of poorly pneumatized mastoids — which were measured planimetrically — among our SOM cohort. This was found in adult SOM ears compared to contralateral healthy ears (4.59 versus 7.88 cm²), as well as when all 102 SOM ears were compared with values of the normal population (5.41 versus 12.9 cm²). This study showed that poorly pneumatized mastoids are a significant risk factor as far as adult SOM is concerned.

KEY WORDS — chronic otitis media, eustachian tube, mastoid, secretory otitis media.

The clinical features of secretory otitis media (SOM) in children have been studied and reported extensively,[1] but as yet there are no reports describing its special character in adults. In children, it has been demonstrated that a negative correlation exists between chronic SOM and the amount of mastoid pneumatization.[2-8] However, since children's mastoid pneumatization has not yet matured, it is impossible to establish whether the small mastoid was predisposed to SOM or whether the long-standing SOM caused the mastoid to stay hypopneumatized. The purpose of this study was to assess the relationship between SOM and mastoid pneumatization in a population of adults whose mastoids had matured many years previously, prior to the beginning of the study.

MATERIALS AND METHODS

Between January 1988 and July 1993, 72 adult patients treated for SOM were enrolled in this study. Only those patients in whom SOM was a recent episode during their adult life were enrolled in this study. Patients with middle ear effusion associated with barotrauma or carcinoma of the nasopharynx were excluded. Patients who presented on first examination with atelectasis or middle ear operations, which would indicate previous protracted middle ear disease, were excluded as well. All the patients were adults, and their ages ranged from 14 to 80, with an average of 49 years. Medical history, otoscopy, nasopharyngeal inspection, and measurement of the extent of mastoid pneumatization were performed by the senior author (J.S.). All patients had had repeated audiograms and tympanometry, but the audiologic features, treatment, and course of the disease will not be discussed here. The extent of the mastoid pneumatization was measured planimetrically by Schuller's projections (Fig 1), a method that has been generally adopted as representing the relative extent of the mastoid pneumatization.[3,6,8-11]

RESULTS

Recent Medical History. Upper respiratory tract infection or acute otitis media (AOM) was reported by 45 patients (63%). Twenty-four patients (33.3%) denied having recently had such an episode, while 3 patients were uncertain.

Nasopharyngoscopy. One patient presented a papilloma originating from the vomer, and 1 patient a

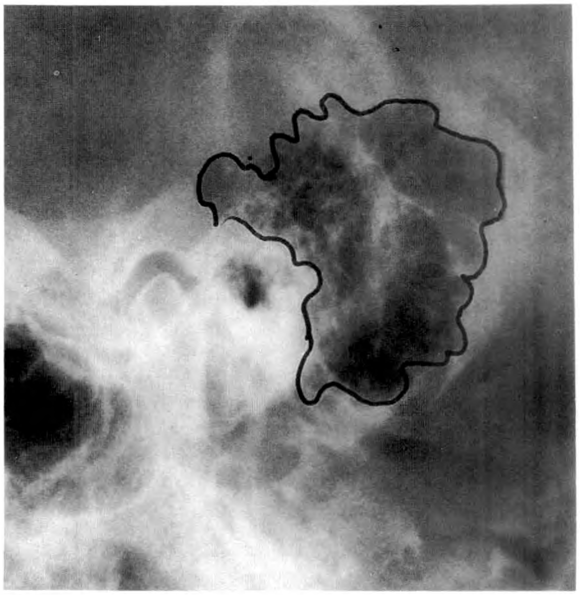

Fig 1. Planimetric demarcation of mastoid pneumatization.

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SUMMARY OF PNEUMATIZATION VALUES FOR SEVERAL SUBGROUPS OF 102 EARS

	Mean (cm^2)	*SD (cm^2)*	*No. of Ears*	*Student's* t *Statistic*	df	*p*
Gender						
Male	5.46	6.80	50			
Female	5.36	5.79	52	0.075	100	.940
Bilateral SOM						
Right ear	6.23	6.59	30			
Left ear	5.86	5.92	30	0.592	29	.558
Unilateral SOM						
Ear with SOM	4.59	6.34	42			
Ear without SOM	7.88	6.40	42	−3.241	41	.001

SOM — secretory otitis media.

visible adenoidal pad in the nasopharynx. In the rest, ie, in 97.2% of patients, the nasopharynx was clear and contained no masses or visible pus.

Gender and Bilaterality of Ears With SOM and Mastoid Pneumatization. We found no significant difference between the mastoid sizes of males (50 ears with a mean of 5.46 cm^2 and an SD of 6.80 cm^2) and females (52 ears with a mean of 5.36 cm^2 and an SD of 5.79 cm^2).

Among the 72 patients, 30 (41.6%) had bilateral SOM and 42 had unilateral SOM, for a total of 102 ears. There was a nonsignificant difference in the mastoids' size between the two ears with SOM when appearing bilaterally: 6.23 and 5.86 cm^2 for right and left ears, respectively. The correlation coefficient was $r^2 = .74$, with 15 of 30 patients having equal pneumatization levels in both ears. In the group with unilateral SOM the mean pneumatization levels were 4.59 cm^2 for the ears with SOM and 7.88 cm^2 for the ears without SOM. The difference was highly significant ($p < .001$). The values are summarized in the Table.

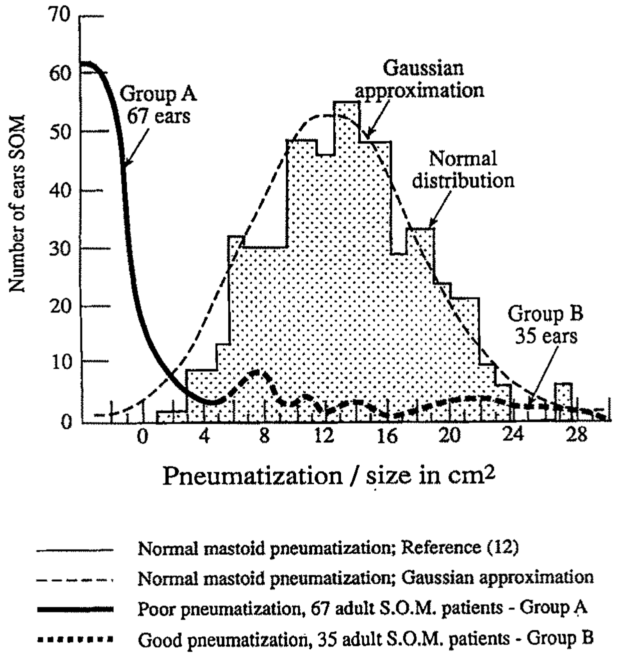

Fig 2. Distribution of pneumatization: normal material (thin line),[12] Gaussian approximation for normal material (dashed line), and patients with secretory otitis media (SOM) from this study (thick line, group A; thick broken line, group B).

Comparison Between Pneumatization Means of SOM and Normal Population. The distribution of the size of the air cell system in our sample of 102 adult ears with SOM had a mean of 5.41 cm^2 and an SD of 6.31 cm^2. The 95% confidence interval for the mean in the appropriate population is between 4.19 and 6.63 cm^2. An empirical study on the size of the air cell system in normal adult patients[12] yielded a sample mean of 12.9 cm^2 and an SD of 5.0 cm^2. Those planimetric results also show an excellent agreement with Diamant's[13] measurements made 40 years ago (a mean of 12.9 cm^2 versus 12.7 cm^2). Both studies show that the distribution of the mastoid size in normal patients is closely approximated by a Gaussian-type curve (Fig 2[12]). The 95% confidence interval for the mean in the healthy population is between 12.1 and 13.7 cm^2. The test statistic for the comparison of the means in the population of SOM versus non-SOM patients is highly significant ($p < .0001$). Thus, on the average, the size of the air cell system in ears with SOM is considerably smaller than both that in the contralateral ear without SOM and that expected in normal ears.

Analysis of Percentiles. The analysis of the distribution of the size of the air cell system in SOM patients reveals an extreme left-hand skewness (Fig 2). The shape of the distribution shows a clear monotonic relationship between poor pneumatization and an increased proportion of SOM occurrence. We have used a rather arbitrary statistical cutoff point, ie, 6 cm^2, to divide ears into two basically different groups. Group A comprises all ears whose mastoid size is 6 cm^2 and lower, and group B comprises all ears with a mastoid above 6 cm^2. The estimate of the probability of all ears' having a pneumatization level

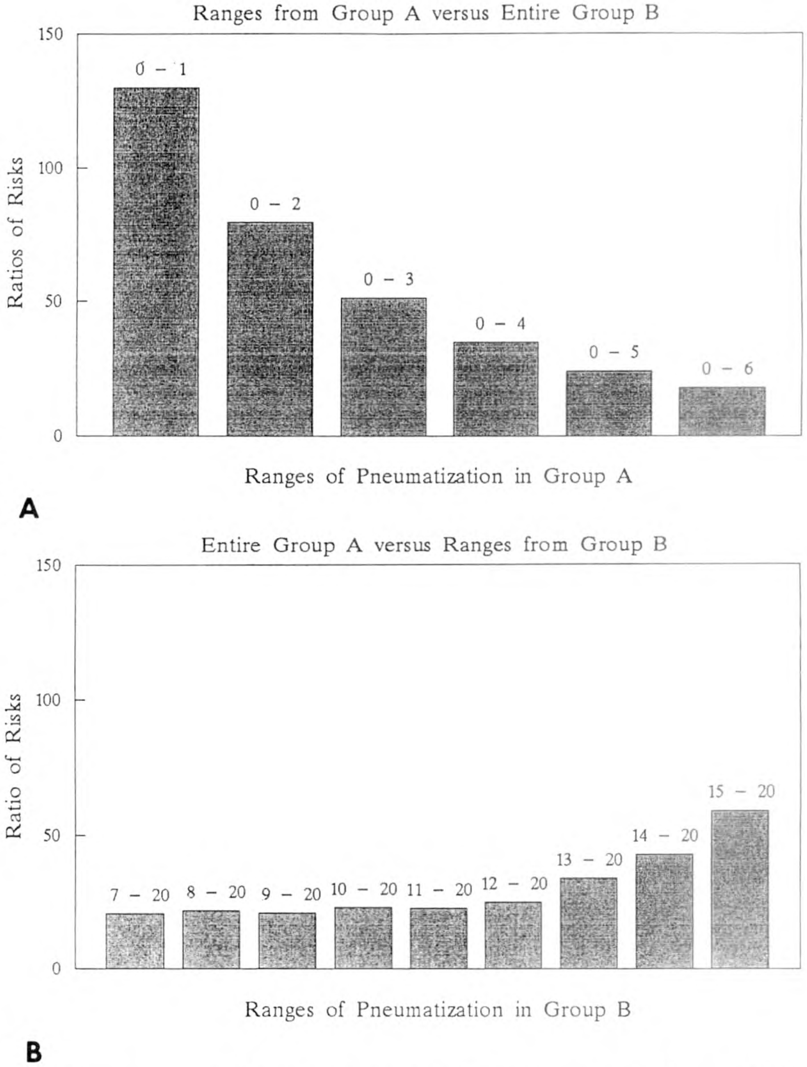

Fig 3. Ratios of risk of SOM occurrence. A) For specific ranges of pneumatization from group A versus entire group B (7 to 28 cm^2). B) For entire group A (0 to 6 cm^2) versus specific ranges of pneumatization from group B. Note that there is approximately equal decreased risk in group B between 7 and 12 cm^2 versus group A, and even further decrease in risk for higher pneumatization, ie, above 13 cm^2.

of 6 cm^2 and lower is 9.7% in normal material versus 65.7% in the ears with SOM in our study. Again, the difference in proportions is highly significant ($p < .0001$). We can conclude that in the adult population of ears with SOM, the proportion of cases with poorly pneumatized mastoids is much higher than in the population of normal ears.

Estimates of SOM Occurrence. The direct assessment of the probability of occurrence of SOM in the general population, given that an individual has a certain level of pneumatization, requires extensive epidemiologic studies. However, interesting enough, while we cannot estimate directly the rates of occurrence of SOM in the population at large, we can assess the ratio of the risks in two populations with different levels of pneumatization. The computations are based on the Bayes theorem (see Appendix[12]) and use both our data and the previously published data on the normal distribution of pneumatization.[12] In particular, we can assess the ratios of the risks of SOM for specific levels of pneumatization in one group (say, below 4 cm^2 in group A) versus the population in the entire other group (say, all group B, ie, above 6 cm^2). Similarly, we can assess the ratios of the SOM risks for levels of pneumatization above each value in group B (say, above 8 cm^2) versus the entire population in group A, ie, less than or equal to 6 cm^2. Those ratios of risk are presented in Fig 3, where it can be seen that a person whose air cell system is less than or equal to 2 cm^2 has a 79 times *higher* probability of an SOM occurrence than a person in group B. From Fig 3B, we see that a person with a pneumatization level larger than 10 cm^2 has about a 23 times *lower* probability of SOM occurrence than a person in group A, and so on. The ratio of risk between each of the two entire groups (A versus B) is 18. Note that there is an approximately constant 21 times higher risk for SOM occurrence in group A as a whole versus the segment of group B in which the values are between 7 and 12 cm^2.

DISCUSSION

The cause of SOM in adults seems to be similar to that in children, in that 63% of the adult patients in this study reported that the onset of their condition was related to an upper respiratory tract infection, and this is also usually the case with children.[14,15] The impression is that during local winter "epidemics" of upper respiratory tract infections, there is a marked rise in incidence of SOM, not only in children, but in adults as well. This inflammatory background corresponds well to the histologic picture, biochemical character, and bacteriological studies of SOM.[1,10]

In 97.4% of the adult patients in this study, the nasopharynx was clear, thus ruling out obstruction as a significant etiologic factor.

A striking feature of this study was the relatively large number of adult patients in whom the mastoid on the affected side was poorly pneumatized. In 65.7% of the ears in this study, the pneumatization level was 6 cm^2 and less, versus 9.7% of the normal adult population[12] (Fig 2). Furthermore, we have estimated the ratios of risks for SOM occurrence for persons with different levels of pneumatization. The analysis clearly indicates that *prior* poor pneumatization of the mastoid can be considered a very significant risk factor in the pathogenesis of SOM in adults. The statistical analysis suggests that persons with pneumatization levels of less than or equal to 6 cm^2 (defined as group A in our study) are 18 times more prone to acquire SOM than persons with better pneumatization (group B). The ratios of risk do not seem to change significantly for various sizes of the air cell system within group B. Thus, for example, the ratio of SOM occurrence in group A versus the persons with 7 cm^2 or more does not differ significantly from the ratio obtained when group A is compared, say, versus persons with 12 cm^2 or more. This seems to provide further justification for the selection of 6 cm^2 as a first cutoff point between groups. A further decrease in the risk of acquiring SOM seems to occur for values of pneumatization larger than the mean of the normal population, ie, larger than 13 cm^2 (Fig 3B). We emphasize, however, that the information from a larger sample size may indicate that the division into groups can be better served by a slightly different cutoff point (or cutoff points) than that defined in this study. Nevertheless, the overall qualitative results are strongly supported by the analysis of this study: the risk of SOM occurrence depends on the pneumatization level — not necessarily in a strictly monotonic manner, but rather as a step function. The risk for SOM occurrence increases as we descend from one level of pneumatization to the other. An obvious conclusion from this study is that SOM in adults is more likely to occur in ears with poorly pneumatized mastoids than in ears with well-pneumatized mastoids.

This study does not concern itself with the mode and pathogenesis of the original formation of a small or a large mastoid pneumatization. All our patients were adults, and their mastoids had already formed to their final size irrespective of what happened to their ears in infancy and childhood. The scanty pneumatization found by us to *precede* SOM may be viewed as a risk factor and, therefore, a likely pathogenetic factor, as previously pointed out by Diamant et al[3,11] and Flisberg and Zigmoid.[9]

The way in which the pneumatic system influences the pathogenesis of SOM may be related to its function as a middle ear physiologic pressure buffer.[16-19] The buffer mechanism operates by virtue of the fact that the volume occupied by the pneumatic space shares in any pressure aberrations, whether positive or negative, of the middle ear cleft, according to Boyle's law. Therefore, the larger the pneumatization, the more efficient is its pressure-equalizing potential. This is also borne out by Holmquist's[19] early studies and the excellent study of Sedeberg-Olsen et al[20] in which the actual size of the middle ear cleft was shown to be related to the prognosis of SOM in children.

Children, in whom the pneumatization system is not yet fully developed, may also be described as a poorly pneumatized group. On the way to puberty, the mastoid system usually matures and pneumatization increases, the progress of many atelectatic conditions is halted or reversed, and the middle ear may revert to normal.[21,22] During growth, most children pass from pneumatization group A to group B — some more slowly than others.

The entire syndrome of chronic otitis media is associated with a middle ear negative pressure, which was widely believed to be secondary to obstruction, narrowing, or other insufficiency of the eustachian tube. The theory of organic aberration of the tubes as an etiologic factor has, however, been disproved.[18,23-26] The phenomenon of middle ear negative pressure probably involves a complex and multifactorial process in which obstruction of the tube by mucus streaming out of the middle ear,[18] the size of the mastoid pneumatization, and fluctuation of gas diffusion into and from the circulation all play a role.[27]

REFERENCES

1. Sadé J. The clinical picture. In: Sadé J, ed. Secretory otitis media and its sequel. New York, NY: Churchill and Livingstone, 1979:1-11.

2. Lumio JS. Contribution to the knowledge of chronic adhesive otitis. Acta Otolaryngol (Stockh) 1951;39:196-209.

3. Diamant M, Rubensohn G, Walander A. Otosalpingitis and mastoid pneumatization. Acta Otolaryngol (Stockh) 1958;49:381-8.

4. Goto T, Kaieda T. The relation of the middle ear pneumatization and the catarrhal otitis media. J Otorhinolaryngol Soc Jpn 1958;61:897-9.

5. Block C, Feustein SS. Serous otitis media and clinical manifestations. Mt Sinai J Med 1976;43:270-2.

6. Sadé J, Hadas E. Prognostic evaluation of SOM as function of pneumatization. Eur Arch Otorhinolaryngol 1979;225:39-44.

7. Hussl B, Welzh-Mueller K. Secretory otitis media and mastoid pneumatization. Ann Otol Rhinol Laryngol [Suppl] 1980;89(suppl 68):79-82.

8. Tos M, Stangerup R. Mastoid pneumatization in secretory otitis. Acta Otolaryngol (Stockh) 1984;98:110-8.

9. Flisberg K, Zigmoid M. The size of the mastoid air cell system: planimetry — direct volume determination. Acta Otolaryngol (Stockh) 1965;60:23-9.

10. Sadé J. The biochemical aspect. In: Sadé J, ed. Secretory otitis media and its sequel. New York, NY: Churchill and Livingstone, 1979:144-54.

11. Diamant M. Chronic otitis. Basel, Switzerland: S Karger, 1952:52-69.

12. Sadé J, Shatz A, Kremer S, Levit I. Mastoid pneumatization in otosclerosis. Ann Otol Rhinol Laryngol 1989;98:451-4.

13. Diamant M. Pneumatization of the mastoid bone. J Laryngol Otol 1958;72:343-64.

14. Hinchcliffe R. Epidemiological aspects of otitis media. In: Glorig A, Gervin KD, eds. Otitis media. Springfield, Ill: Charles C Thomas, 1972:36-43.

15. Sadé J. The natural history of secretory otitis media. In: Sadé J, ed. Secretory otitis media and its sequel. New York, NY: Churchill and Livingstone, 1979:89-101.

16. Richards SH, O'Neill G, Wilson F. Middle ear pressure variations during general anesthesia. J Laryngol Otol 1982;96:883-92.

17. Sadé J. Mastoid pneumatization and middle ear aeration. Eur Arch Otorhinolaryngol 1992;249:301-4.

18. Sadé J. The nasopharynx, eustachian tube and otitis media. J Laryngol Otol 1994;108:95-100.

19. Holmquist J. Aeration in chronic otitis media. Clin Otolaryngol 1978;8:279-84.

20. Sedeberg-Olsen JF, Sedeberg-Olsen AE, Jensen AM. The prognostic significance of the air volume in the middle ear for the tendency to recurrence of secretory middle ear condition. Int J Pediatr Otorhinolaryngol 1983;5:179-87.

21. Sadé J. Treatment of retraction pockets and cholesteatoma. Eur Arch Otorhinolaryngol 1993;250:193-9.

22. Sadé J. The correlation of middle ear aeration with mastoid pneumatization. Eur Arch Otorhinolaryngol 1992;249:301-4.

23. Sadé J, Luntz M. Eustachian tube lumen: comparison between normal and inflamed specimens. Ann Otol Rhinol Laryngol 1989;98:630-4.

24. Sadé J, Luntz M. Adenoidectomy in otitis media. A review. Ann Otol Rhinol Laryngol 1991;100:226-31.

25. Sadé J, Luntz M, Yaniv E, Yarovitzki I. Eustachian tube lumen in chronic otitis media. Am J Otol 1986;6:439-42.

26. Palva T, Karya J. Relationship of pre-operative eustachian tube function to tympanoplasty. Acta Otolaryngol (Stockh) [Suppl] 1970(suppl 263):25-8.

27. Sadé J, Luntz M. Dynamic measurement of gas composition in the middle ear. Acta Otolaryngol (Stockh) 1993;113:353-7.

APPENDIX

Let us define

$X =$ The size of the air cell system of a randomly selected person.

$P(SOM) =$ Marginal probability of occurrence of SOM.

$P(X \leq a) =$ Marginal probability that the size of the air cell system of a randomly selected person is less than or equal to *a*.

$P(X > b) =$ Marginal probability that the size of the air cell system of a randomly selected person is greater than *b*.

$P(SOM \mid X \leq a) =$ Conditional probability of occurrence of SOM in a person whose size of the air cell system is less than or equal to *a*.

$P(SOM \mid X > b) =$ Conditional probability of occurrence of SOM in a person whose size of the air cell system is greater than *b*.

$P(X \leq a \mid SOM) =$ Conditional probability that the size of the air cell system of a person with SOM is less than or equal to *a*.

$P(X > b \mid SOM) =$ Conditional probability that the size of the air cell system of a person with SOM is greater than *b*.

Then we have

$$P(SOM \mid X \leq a) = P(X \leq a \mid SOM) \times P(SOM)/P(X \leq a)$$

and

$$P(SOM \mid X > b) = P(X > b \mid SOM) \times P(SOM)/P(X > B)$$

Therefore,

$$P(SOM \mid X \leq a)/P(SOM \mid X > b) = \{P(X < a \mid SOM)/P(X > b \mid SOM)\}/\{P(X \leq a)/P(X > b)\}$$

For example, for $a = b = 6$, we estimate from our study the first term as (67/35) = 1.91. Now, from our normal data[12] we estimate the second term as (0.097/0.903) = 0.107. The ratio of the two terms, ie, (1.914/0.107) = 17.8, is the estimated ratio of rates of occurrence of SOM in persons whose size of the air cell system is less than 6 cm^2 versus persons with better pneumatization.

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ROLE OF SUBSTANCE P IN THE VASCULAR RESPONSE OF NASAL MUCOSA IN NASAL ALLERGY

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The effects of topically administered substance P (SP) on nasal blood flow and nasal airway resistance (NAR) were evaluated in 11 subjects with perennial nasal allergy. The change in NAR induced by SP was compared with those induced by nasal challenge with histamine, leukotriene D4 (LTD4), and antigen. In doses ≥16 nmol, SP caused a significant increase of nasal blood flow within 5 minutes that lasted for less than 20 minutes. In doses ≥16 nmol, SP caused a dose-dependent, short-lasting, significant increase in NAR. The magnitude of the increase in NAR was LTD4 > SP > histamine when compared on a molar basis. Our results may suggest that SP released from C fiber terminals is partially involved in an early nasal vascular response after antigen challenge by acting on adjacent vascular smooth muscle to cause a transient vasodilatation of both resistance and capacitance vessels only while sensory stimulation persists in subjects with nasal allergy.

KEY WORDS — mucosal blood flow, nasal airway resistance, nasal allergy, substance P.

INTRODUCTION

In most species, including humans, a subpopulation of the trigeminal neurons innervating the nasal mucosa is substance P (SP)– and calcitonin gene–related peptide (CGRP)–immunoreactive (IR) and is located both beneath and within the epithelium, as well as around the arterioles, venules, sinusoids, and nasal glands.[1-3] These fibers belong to the C fiber group, which transmits the nociceptive sensations of itch, burning, and pain.[4] Capsaicin extracted from hot pepper affects these SP- and CGRP-IR nerve terminals to cause release of SP and CGRP, resulting in a long-lasting nonadrenergic and noncholinergic dilatation of resistance and capacitance vessels of the nasal mucosa in cats.[5] Antidromic electrical stimulation of the maxillary division of the trigeminal nerve in the sympathectomized cat causes atropine-resistant and hexamethonium-resistant dilatation of resistance and capacitance vessels with increased vascular permeability in cat nasal mucosa.[6] The flare response that is observed surrounding a wheal when human skin is injected with histamine is considered to be induced by the axon reflex, which is significantly inhibited by capsaicin pretreatment.[7] In subjects with nasal allergy, histamine released from mast cells on antigen-antibody reaction in the nasal mucosa is a major chemical mediator in causing sneezing by stimulating the SP- and CGRP-IR nerve terminals distributed beneath and within the epithelium.[8] It may be possible that histamine may also cause nasal vascular reactions via the axon reflex mediated by SP and CGRP, besides causing potent direct vascular effects. The possible role of SP and other sensory peptides in the development of hyperreactive symptoms in airway allergy has been hypothesized by some authors.[9,10] However, the response of the human nasal vasculature to SP in vivo, and particularly the possible role of SP via axon reflex in pathogenesis of nasal mucosal swelling, are still controversial.[11-16] The present study was performed to evaluate the possible role of SP via the axon reflex in swelling of the nasal mucosa induced by chemical mediators in subjects with nasal allergy. We have evaluated the effects of exogenous SP on the resistance and capacitance vessels of the nasal mucosa and sneezing in subjects with nasal allergy and have compared them with the effects of histamine, leukotriene D4 (LTD4), and antigen.

SUBJECTS AND METHODS

Subjects. Eleven patients with perennial nasal allergy (4 women, 7 men; age 17 to 28 years) who had more than moderate hyperreactive nasal symptoms according to Okuda's criteria[17] and were allergic to house dust and mites alone, confirmed by skin tests, nasal provocation tests, and radioallergosorbent tests, were enrolled in the study. No drugs were allowed during the 3 weeks preceding the study. All subjects gave informed consent prior to the study.

Study Design. The time schedule of the study is shown in Fig 1. On day 1, the effect of topically administered SP in doses of 8 to 16 nmol on blood

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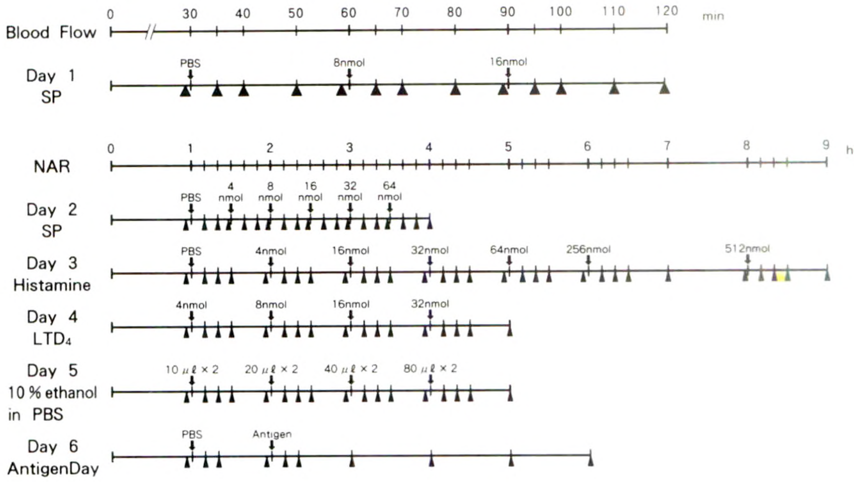

Fig 1. Time schedule of study. ↓ — administration of phosphate-buffered saline (PBS), substance P (SP), histamine, leukotriene D4 (LTD4), 10% ethanol in PBS as diluent for LTD4 and antigen; ▲ — measurement of blood flow or nasal airway resistance (NAR).

flow of the nasal mucosa was evaluated with a laser Doppler flowmeter (Vasamedics BPH-403A, St Paul, Minn). On days 2 to 6, the effects of topical administration of SP (4 to 64 nmol), histamine (4 to 512 nmol), LTD4 (4 to 16 nmol), 10% ethanol in phosphate-buffered saline (PBS) used as a diluent for LTD4, and antigen on the time course of changes of nasal airway resistance (NAR) were measured by means of active posterior rhinomanometry (Nihon Kohden, MRP2100, Japan).

Variations of frequency of sneezing as a parameter to evaluate the degree of sensory stimulation after nasal challenge with each substance were also recorded. Synthetic SP (Peptide Institute Inc, Japan) and histamine diphosphate (Nakarai Chemical Ltd, Japan) were dissolved in PBS (pH 7.4, 286 mosm). Leukotriene D4 (Sigma) stocked in 100% ethanol was dissolved in PBS on use. Each measurement was performed on 6 different days separated by at least 7 days.

Evaluation of Response of Blood Flow in Nasal Mucosa to SP. After the subjects rested in the supine position for 30 minutes, a piece of filter paper (5 × 10 mm) soaked with diluent (PBS, 20 μL), 8 nmol, and 16 nmol of SP dissolved in 20 μL of PBS was unilaterally applied for 5 minutes at 30-minute intervals to the median surface of the inferior turbinate mucosa near its anterior tip. Change of blood flow was measured by fixing the tip of a fiberoptic probe of 3 mm diameter on the nasal mucosa where test solutions were applied, just before application and at 5, 10, 20, and 30 minutes after removal of the filter paper soaked with test solution.

Evaluation of Changes of NAR Induced by SP, Histamine, LTD4, and Antigen. After the subjects remained in the room for 30 minutes, following the time schedule shown in Fig 1, on day 2, PBS and 4, 8, 16, 32, and 64 nmol SP dissolved in 20 μL × 2 of PBS per bilateral nasal cavities were instilled at 30-minute intervals on the inferior turbinate mucosa near the anterior tips bilaterally through a micropipette under direct vision. During the study the subjects were in a sitting position. After the subjects blew their noses gently, NAR was measured at 10, 20, and 30 minutes after administration of test solutions. Resistance at the inspiratory flow 0.15 L/s was calculated and was expressed as centimeters of water per liter per second.

On day 3, NAR was measured just before and at 10, 20, 30, and 60 minutes after topical administration of 20 μL × 2 of PBS and histamine solution in doses of 4, 16, 32, 64, 256, and 512 nmol dissolved in 20 μL × 2 of PBS per bilateral nasal cavities at 1-hour intervals. On day 4, NAR was measured just before and at 10, 20, 30, and 60 minutes after topical administration of LTD4 solution in doses of 4, 8, and 16 nmol dissolved in 20 μL × 2, 40 μL × 2, and 80 μL × 2 10% ethanol in PBS, respectively, at 1-hour intervals. On day 5, NAR was measured before and after topical administration of 20 μL × 2, 40 μL × 2, and 80 μL × 2 10% ethanol in PBS following the same time schedule as day 5. On day 6, antigen challenge was performed with an antigen disc that contained 12.5 μg freeze-dried dust mites per disc (Torii Pharmaceutical Ltd, Japan) on the inferior turbinate bilaterally, and NAR was measured just before and at $^{1}/_{6}$, $^{1}/_{3}$, $^{1}/_{2}$, 1, 2, 3, and 4 hours following antigen challenge.

Statistical Analysis. The data are expressed as mean and standard deviation. Statistical analysis was performed with a one-way analysis of variance, and p values less than 5% were considered significant.

RESULTS

Change of Blood Flow in Resistance and Exchange Vessels of the Nasal Mucosa Induced by

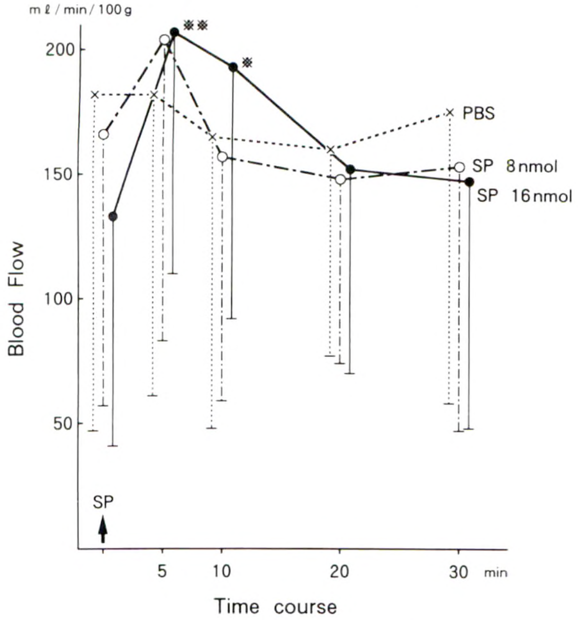

Fig 2. Variation of blood flow in nasal mucosa induced by SP measured by Doppler flowmeter. Sixteen nanomoles of SP caused significant increase in blood flow that lasted for less than 20 minutes. * — p < .05, ** — p < .01.

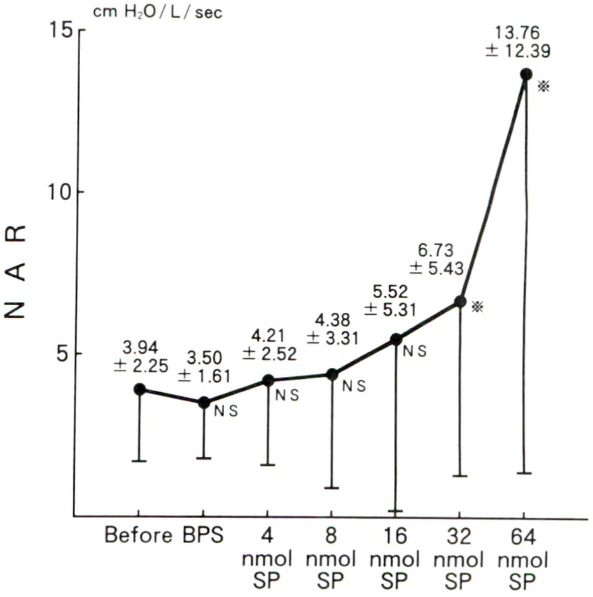

Fig 3. Dose-response relations for increase of NAR induced by SP. Nasal airway resistance was measured 10 minutes after application of SP, which caused dose-dependent significant increase in NAR. * — p < .05, NS — no significant difference.

Topical Administration of SP. Although diluent did not cause a significant change in blood flow, 8 nmol of SP caused an increase in blood flow, although not significant statistically, of 24% over the base level, and 16 nmol of SP caused a statistically significant increase in blood flow — 55% ($p < .01$) over the base level at 5 minutes and 44% ($p < .05$) at 10 minutes — which returned to baseline in less than 20 minutes (Fig 2).

Changes of NAR Induced by Nasal Challenge of SP, Histamine, LTD4, and Antigen. Whereas diluent did not cause any significant change in NAR, challenges with SP, histamine, and LTD4 caused a dose-dependent increase in NAR. Increases in NAR induced by 32 and 64 nmol of SP were 70% and 249% of baseline NAR, respectively, which were significant statistically (Fig 3). The duration of increase in NAR induced by 64 nmol SP was less than 20 minutes in 9 of 11 subjects (Fig 4A). Six of 11 subjects developed facial cutaneous flushing with a sensation of warmth in the cheeks and palpitations within 1 to 2 minutes following administration of 64 nmol of SP, which lasted for less than 1 to 2 minutes. Variations of NAR induced by topical administration of histamine and LTD4 are shown in Fig 4B,C. Challenges with the active substances resulted in a significant increase from baseline at doses of ≥8 nmol for LTD4, ≥32 nmol for SP, and 64 nmol for histamine. The relative ordering of the substances with regard to the magnitude of the increase in NAR was LTD4 > SP > histamine when compared on a molar basis (32 nmol). The magnitude of increase in NAR induced by SP in doses of 32 nmol was almost the same as that induced by histamine in doses of 64 to 256 nmol. After antigen challenge, the NAR increase proceeded gradually, reaching a peak at 1 hour. Although it tended to return to baseline NAR after 1 hour, the increase of NAR at 2 hours was still significant compared to baseline NAR (Fig 5B). In contrast, the duration of a significant increase of NAR induced by SP in doses ≤64 nmol was less than 20 minutes, and those induced by LTD4 in doses of ≤16 nmol and histamine of ≤64 nmol were less than 1 hour.

Relationship Between Sneezing as Parameter for Evaluating Strength of Sensory Stimulation and Change of NAR Observed on Nasal Challenge With Histamine and Antigen. The numbers of sneezes induced by SP and LTD4 were not significantly different from those induced by their diluents. Figure 6 shows dose-response relationships for number of sneezes and for percent change of NAR induced by topical administration of various doses of histamine. Histamine at a dose of 4 nmol induced a significant number of sneezes in 9 of 11 subjects. However, the same dose of histamine induced a significant increase in NAR in only 1 subject. The threshold level of histamine for causing a significant number of sneezes was 4 nmol, while 64 nmol was required to induce a significant increase in NAR.

When we compared the variation of the number of sneezes with that of the change of NAR induced by

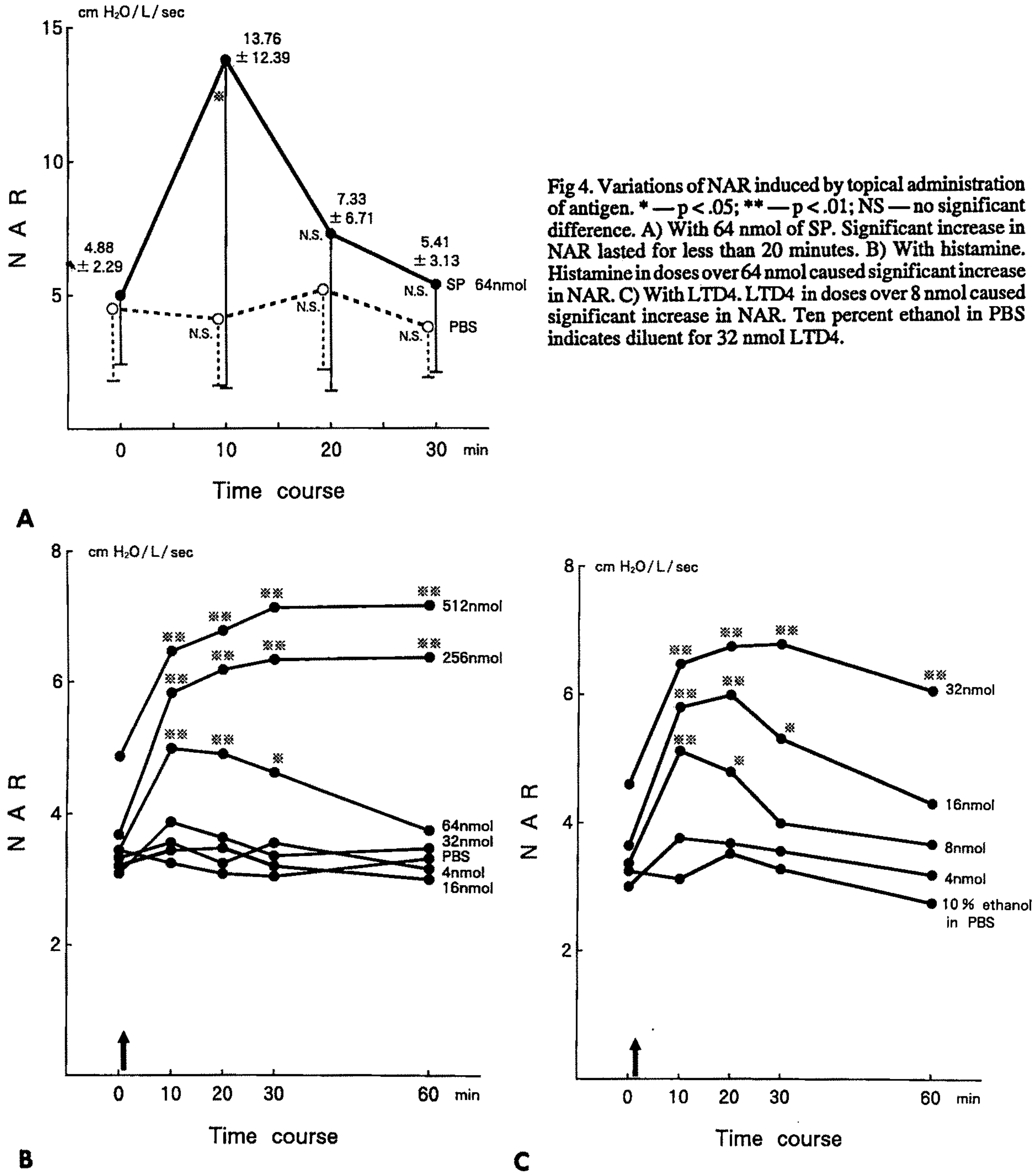

Fig 4. Variations of NAR induced by topical administration of antigen. * — $p < .05$; ** — $p < .01$; NS — no significant difference. A) With 64 nmol of SP. Significant increase in NAR lasted for less than 20 minutes. B) With histamine. Histamine in doses over 64 nmol caused significant increase in NAR. C) With LTD4. LTD4 in doses over 8 nmol caused significant increase in NAR. Ten percent ethanol in PBS indicates diluent for 32 nmol LTD4.

antigen challenge, an apparent difference was observed between them. Sneezing started within 5 minutes after antigen challenge in most subjects and lasted for less than 10 minutes, while NAR increased gradually and reached a peak after 1 hour (Fig 5).

DISCUSSION

Vascular responses measured by the laser Doppler flowmeter indicate changes of blood flow in resistance and exchange vessels in the nasal mucosa.[18] An increase of NAR reflects dilatation of capacitance vessels and/or enhanced vascular permeability in the nasal mucosa. The blood flow of the nasal mucosa measured by the laser Doppler flowmeter increased statistically significantly within 5 minutes after administration of 16 nmol of SP and returned to the base level in less than 20 minutes. Substance P in doses over 32 nmol induced a significant dose-dependent increase in NAR. The magnitude of increase in NAR induced by SP in doses of 32 nmol was almost equal to that induced by LTD4 in doses of 8 to 16 nmol and was almost the same as that induced by histamine in doses of 64 to 256 nmol. The ratio of the molecular weights of each substance is 1,347:496:307 for SP: LTD4:histamine.

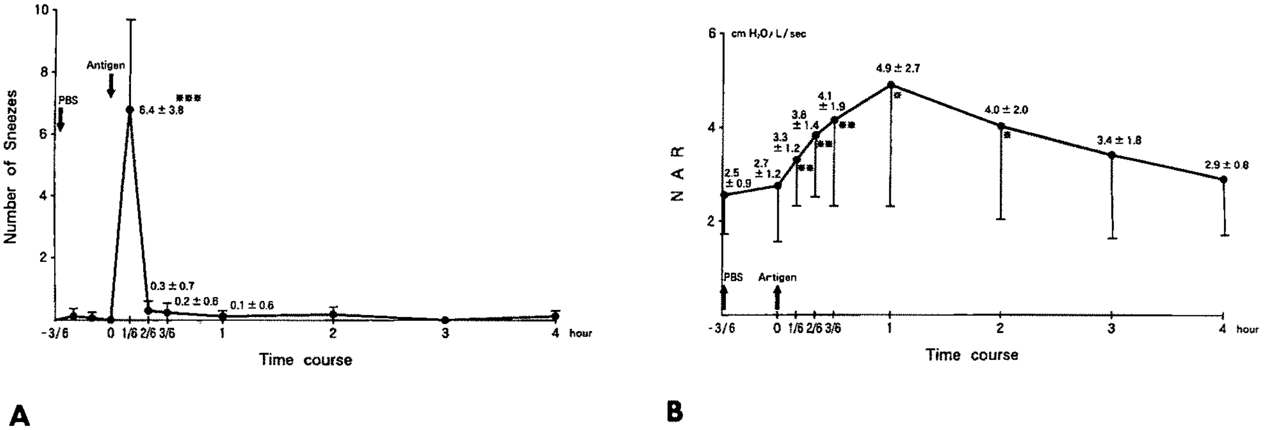

Fig 5. Variations of A) frequency of sneezes and B) NAR induced by antigen challenge on nasal mucosa. * — $p < .05$, ** — $p < .01$, *** — $p < .001$. Sneezing started within 5 minutes after antigen challenge in most subjects and continued for less than 10 minutes, while NAR increased gradually and reached peak after 1 hour.

The amounts of histamine and LTD4 in the nasal lavages at 10 minutes after antigen challenge were 162×10^{-3} nmol and 1.14×10^{-3} nmol, respectively,[19] and the amount of SP in nasal lavages after antigen challenge was reported to be about 0.08×10^{-3} nmol.[14] In view of these large differences in the amount of each of these endogenous substances[15,19-21] in nasal lavages obtained after antigen challenge in subjects with nasal allergy, and possible large differences in absorption and degradation rates of these exogenous and endogenous substances in and on the nasal mucosa, it is difficult to define the comparative importance of these substances in the pathogenesis of mucosal swelling in nasal allergy. Compared to the sustained increase of NAR induced by antigen, LTD4, and histamine, the increase in NAR induced by SP in doses almost causing systemic side effects was rather short-lasting. Histamine is considered to cause sneezing by stimulating C fiber terminals distributed in and under the nasal epithelium.[5,8] Stimulation of C fibers by histamine may cause nasal vascular responses via the axon reflex. However, although histamine in a dose of 4 nmol was sufficient to cause sneezing in 9 of 11 subjects, a significant increase in NAR induced by the same dose was observed in only 1 subject. The present study may indicate that SP released from SP-IR terminals distributed adjacent to the vascular smooth muscle in response to sensory stimulation may be partially involved in an early vascular reaction after antigen challenge by causing relaxation of the vascular smooth muscle resulting in a short-lasting dilatation of resistance and also capacitance vessels with short latency in the nasal mucosa only while sensory stimulation persists in subjects with nasal allergy. Substance P injected intradermally in humans has been demonstrated to produce a dose-dependent wheal and flare reaction.[22,23] As the flare reaction was abolished by pretreatment with H1 receptor antagonist, this was considered to be caused by histamine released from mast cells.[22,24] In the present study, topically administered SP induced neither sneezing nor nasal itching. This is consistent with previous reports, which showed that exogenous SP in doses of 10 to 80 nmol[11] and 20 to 160 nmol[14] on the nasal mucosa did not cause an increase of histamine in nasal lavages. The observed vascular responses induced by SP in the present study were considered to be direct effects of SP on the nasal vasculature without involvement of mast cell degranulation. Mucosal swelling observed after antigen challenge is

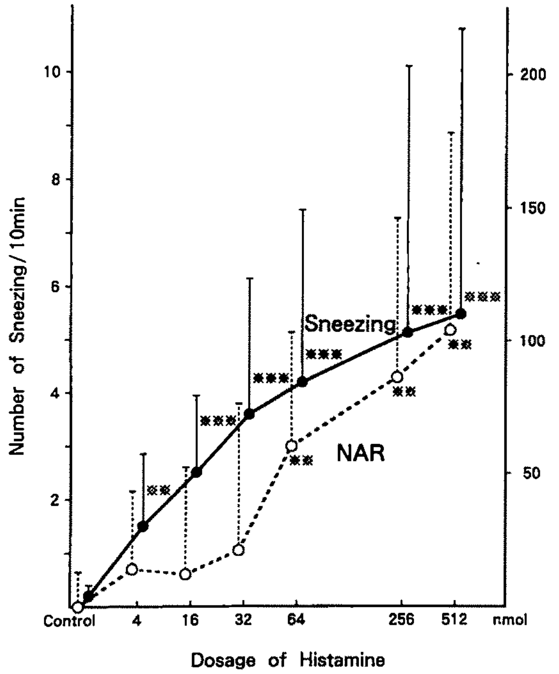

Fig 6. Dose-response relations for number of sneezes per 10 minutes (filled circles) and for percent change of NAR in 10 minutes (empty circles) after topical application of various doses of histamine. Threshold level of histamine to cause significant number of sneezes was 4 nmol, but 64 nmol was required to induce significant increase in NAR. ** — $p < .01$, *** — $p < .001$.

caused by dominant plasma protein extravasation together with vasodilatation. Antidromic stimulation of the trigeminal nerve in the cat[6] and topical administration of SP in doses of 10 to 80 nmol on the nasal mucosa in subjects with nasal allergy[11] have also been reported to cause an increase in plasma protein extravasation and inflammatory cellular responses besides vasodilatation. However, judging from the fairly rapid time course of changes in NAR induced by SP compared to those induced by antigen, a short-lasting increase in NAR with short latency induced by SP in the present study is assumed to reflect mainly dilatation of capacitance vessels in the nasal mucosa. There are diverse opinions regarding the effects of SP on nasal blood flow[25] and NAR.[11-13,16] These differences must be due to different SP dosages and different methods of administration. In the development of mucosal swelling observed after antigen challenge, various vasoactive substances are involved, including those from basophilic cells, C fiber terminals, parasympathetic nerve terminals, and others. To evaluate the comparative importance of SP further, we need a pure and potent SP antagonist available for clinical study.

REFERENCES

1. Lundblad L, Lundberg JM, Brodin E, Anggärd A. Origin and distribution of capsaicin-sensitive substance P–immunoreactive nerves in the nasal mucosa. Acta Otolaryngol (Stockh) 1983;96:485-93.
2. Stjärne P, Lundblad L, Anggärd A, Hökfelt T, Lundberg JM. Tachykinins and calcitonin gene–related peptide: coexistence in sensory nerves of the nasal mucosa and vascular effects compared to capsaicin. Cell Tissue Res 1989;256:439-46.
3. Uddman R, Malm L, Sundler F. Substance P–containing nerve fibers in the nasal mucosa. Arch Otorhinolaryngol 1983;238:9-16.
4. Holtzer P. Local effector functions of capsaicin-sensitive sensory nerve endings: involvement of tachykinins, calcitonin gene–related peptide and other neuropeptides. Neuroscience 1988;24:739-68.
5. Lundblad L. Protective reflexes and vascular effects in the nasal mucosa elicited by activation of capsaicin sensitive substance P–immunoreactive trigeminal neurons. Acta Physiol Scand Suppl 1984(suppl 529).
6. Lundblad L, Anggärd A, Lundberg JM. Effects of antidromic trigeminal nerve stimulation in relation to parasympathetic vasodilatation in cat nasal mucosa. Acta Physiol Scand 1983;119:7-13.
7. McCusker MT, Chung KF, Roberts NM, Barnes PJ. Effect of topical capsaicin on the cutaneous response to inflammatory mediators and to antigen in man. J Allergy Clin Immunol 1989;83:118-24.
8. Konno A, Nagata H, Nomoto M, Motosugi H, Terada N. Role of capsaicin-sensitive trigeminal nerves in development of hyperreactive nasal symptoms in guinea pig model of nasal allergy. Ann Otol Rhinol Laryngol 1995;104:730-5.
9. Barnes PJ. Asthma as an axon reflex. Lancet 1986;ii:242-5.
10. Payan DG, Levine JD, Goetzl EJ. Modulation of immunity and hypersensitivity by sensory neuropeptides. J Immunol 1984;132:1601-4.
11. Braunstein G, Fajac I, Lacsonique J, Frossard N. Clinical and inflammatory responses to exogenous tachykinins in allergic rhinitis. Am Rev Respir Dis 1991;144:630-5.
12. Devillier P, Dessanges JF, Rakotosikanaka F, et al. Nasal response to substance P and methacholine in subjects with and without allergic rhinitis. Eur Respir J 1988;1:356-61.
13. Miadonna A, Tedeschi A, Leggieri E, et al. Activity of substance P on human skin and nasal airways. Ann Allergy 1988;61:220-3.
14. Nieber K, Baumgarten C, Witzel A, et al. The possible role of substance P in the allergic reaction, based on two different provocation models. Int Arch Allergy Appl Immunol 1991;94:334-8.
15. Walker KB, Serwonska MH, Valone FH, et al. Distinctive patterns of release of neuroendocrine peptides after nasal challenge of allergic subjects with ryegrass antigen. J Clin Immunol 1988;8:108-13.
16. Wolf G, Loidt D, Saria A, Gamse R. Änderungen des nasalen Volumenstromes nach lokaler Applikation des Neuropeptides Substanz-P und von Capsaicin. Laryngol Rhinol Otol (Stuttg) 1987;66:412-5.
17. Okuda M. Fundamental and clinical studies on nasal allergy. Otologia Fukuoka 1974;20:297-344.
18. Olsson P, Bende M, Ohlin P. The laser Doppler flow meter for measuring microcirculation in human nasal mucosa. Acta Otolaryngol (Stockh) 1985;99:133-9.
19. Terada N, Ando H, Ito E, et al. Nasal allergy and leukotriene — kinetics of peptide leukotrienes and inflammatory cells in nasal lavage fluid after antigen challenge. J Otolaryngol Jpn 1989;92:1337-44.
20. Chaen T, Watanabe N, Mogi G, Mori K, Takeyama M. Substance P and vasoactive intestinal peptide in nasal secretions and plasma from patients with nasal allergy. Ann Otol Rhinol Laryngol 1993;102:16-21.
21. Tonnesen P, Hindberg I, Schaffalitzky de Mackadell OB, Mygind N. Effect of nasal allergen challenge on serotonin, substance P and vasoactive intestinal peptide in plasma and nasal secretions. Allergy 1988;43:310-7.
22. Foreman JC, Jordan CC, Oehme P, Renner H. Structure-activity relationship for some substance P–related peptides that cause wheal and flare reactions in human skin. J Physiol (Lond) 1983;335:449-65.
23. Hagermark O, Hokfelt T, Pernow B. Flare and itch induced by substance P in human skin. J Invest Dermatol 1978;71:233-5.
24. Barnes PJ, Brown MJ, Dollery CT, Fuller RW, Heavey DJ, Ind RW. Histamine is released from skin by substance P but does not act as the final vasodilator in the axon reflex. Br J Pharmacol 1986;88:741-5.
25. Yamaguchi M. Mucosal blood volume in the human nose. Measurement with a spectrophotometric analysis. Pract Otol (Kyoto) 1991(suppl 44):1-39.

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OTONEUROLOGICAL EXPERT SYSTEM

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An otoneurological expert system was developed to help collect data and diagnose both central and peripheral diseases causing vertigo. Patient history and otoneurological and other examination results are used in the reasoning process. The case history data can be either mandatory or supportive. Mandatory questions are used to confirm a diagnosis, and conflicting answers are used to reject an unlikely disease. Supportive questions support or suppress a diagnosis, but their presence is not obligatory. The reasoning procedure of the otoneurological expert system scores every question independently for different diagnoses, depending on how well they agree with the symptom entity of a disease. Diagnostic criteria are set for each disease. Graphic displays illustrate the linear and nonlinear correlation between the symptoms and diseases. Emphasis is placed on diminishing the possibility of a wrong decision rather than maximizing the likelihood of reaching only one right decision, so that even rare diseases can be taken into consideration.

KEY WORDS — artificial intelligence, dizziness, otoneurological expert system, vestibular diagnosis.

INTRODUCTION

Vertigo, or dizziness, is a problem confronting physicians in different fields of medicine. Two expert systems have been presented to aid the diagnostic processing of vertigo.[1,2] They both have strong and weak points. For example, although they concentrate only on diseases originating from peripheral causes, the deductive work is mainly based on patient history, and the aid of otoneurological tests has thus far remained unused in the diagnostic work.

Expert systems are used either to aid the diagnostic workup or to guide treatment choice when a diagnosis is known. With the choice of treatment, the causal reasoning mechanisms can be good, but in diagnostic work, where the cause of the disease can be unknown or hypothetical, as it is in Meniere's disease, a rule-based technique may not be suitable. In other fields of medicine several expert programs have been developed. Some systems use epidemiologic information in addition to the rule-based technique to aid diagnostic work.[3] It is well suited to some areas of medical reasoning, but it may sometimes lead to overgeneralization of the most frequent disease so that infrequent diseases are lost in the reasoning.[4]

Our goal was to create an expert system that incorporates even rare diseases into the workup for vertigo. We tried to diminish the risk of obtaining a wrong decision rather than to maximize the possibility of making only one right decision. Reliability is crucial in medical decision making. This otoneurological expert system, called ONE, can suggest several diseases, but the ultimate decision is made by the user.

BACKGROUND OF OTONEUROLOGICAL EXPERT SYSTEM

We planned ONE for use with our data base for vertigo. The system requires a 640-kilobit IBM-compatible personal computer with a videographics array display. The program is written in C-language and has a high-quality graphic user interface. Detailed information about patient history and symptoms and findings in neurotological, audiological, and imaging tests are stored in the data base. The symptoms have been classified into three sets of questions on vertigo, hearing loss, and tinnitus and their provoking factors. The data base offers an optimal means by which to store large amounts of specific information that can be quickly retrieved if needed. This information is collected in the data base interactively with a personal computer or with the use of a questionnaire. The questionnaire is easily filled out by the patient in the waiting room, and it provides sufficient background on a patient's vertigo. ONE is designed to be user-friendly and assists the user in many ways. By asking, the user can obtain additional information about the questions or the system itself. Every screen is accompanied by a help panel that assists the input of information.

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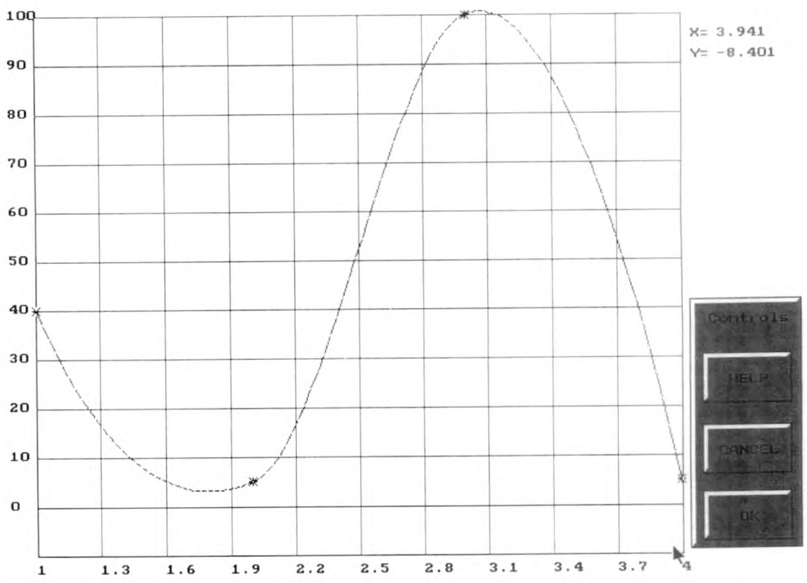


Fig 1. Graphic display of scores for attack duration in Meniere's disease.

Since it is rarely possible to offer all the information that the system asks for, ONE has been constructed to work with incomplete information. The blank answers are taken into consideration in the reasoning process and are included in the reliability margins of the final result.

REASONING

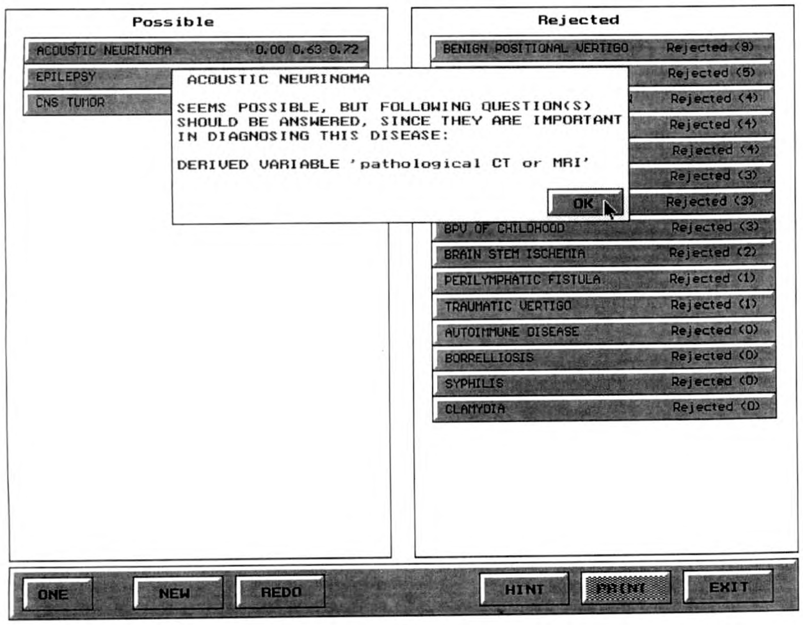
The reasoning procedure of ONE has been implemented with a scoring scheme in which scores are determined for different diseases on the basis of the perceived symptoms and the results of clinical tests performed on the patient.[5] For the reasoning process, patient history and the results of otoneurological tests are collected in the data base (Kentala et al, unpublished observations, 1994). The knowledge base of ONE comprises precise information on how symptoms and otoneurological test results correspond to the diseases incorporated in the system. Certain diagnostic criteria are set for each disease, and they have been taken from the literature and medical journals.[6-10] For example, the full triad is required for diagnosing Meniere's disease. The reasoning resembles the pattern recognition method. The ONE recognizes compatible diseases from the symptoms and signs that are given by the user. There are three partly independent factors used in reasoning: questions, weighted values, and scores.

Questions. Questions are presented in various forms. Their answers can be strings, numbers in different combinations, and logical yes or no values. The numeric values are set separately for each question and can range from 0-4 to 0-100. With this variation we have increased the resolution quantities of the program.

The questions have been divided into mandatory and supportive categories. Mandatory questions require definite affirmative answers for a diagnosis, while supporting questions only suggest a disease. These properties were developed primarily to characterize diseases and secondarily to restrict the size of the search space in reasoning.

Weighted Values. Every question has a weighted value for the possible diseases incorporated into ONE. The weighted values are stored in the knowledge base. They correspond to the significance of the question associated with a certain disease. The weighted values range from –5 to 80, and were experimentally derived from our data. Zero means that the answer does not concern the disease, positive values correlate with the disease, and negative values indicate that a particular disease is unlikely. The higher the weighted value, the more significant the question in the reasoning process for that disease.

Scores. Points are set for every answer possibility for all diseases incorporated into the program. The more evidently some symptom or finding belongs to a certain disease, the more weight is given to this symptom. Graphic display is used in the scoring to illustrate better the linear and nonlinear correlations among the symptoms and diseases. A simple linear correlation can be seen in the strength of nausea with vestibular neuronitis. A more varying correlation is seen with attack duration in Meniere's disease, for which both seconds-long Tumarkin-type attacks and attacks lasting hours give a high score, while intermediately long attacks score much lower (Fig 1). The possible test results in otoneurological tests are displayed graphically for the scoring of each disease. The values for the graphic displays have been developed from information from an otoneurological expert and the literature.



ACOUSTIC NEURINOMA 0.00 0.63 0.72
EPILEPSY
CNS TUMOR

BENIGN POSITIONAL VERTIGO Rejected (9)
Rejected (5)
Rejected (4)
Rejected (4)
Rejected (4)
Rejected (3)
Rejected (3)
BPV OF CHILDHOOD Rejected (3)
BRAIN STEM ISCHEMIA Rejected (2)
PERILYMPHATIC FISTULA Rejected (1)
TRAUMATIC VERTIGO Rejected (1)
AUTOIMMUNE DISEASE Rejected (0)
BORRELLIOSIS Rejected (0)
SYPHILIS Rejected (0)
CLAMYDIA Rejected (0)

```
ACOUSTIC NEURINOMA

SEEMS POSSIBLE, BUT FOLLOWING QUESTION(S)
SHOULD BE ANSWERED, SINCE THEY ARE IMPORTANT
IN DIAGNOSING THIS DISEASE:

DERIVED VARIABLE 'pathological CT or MRI'
```

Fig 2. Result panel with most probable disease.

The maximum number of points is the weighted value of the question. The scores are based on the diagnostic criteria of the disease. The probabilities of diseases do not affect the scoring or reasoning.

Uncertainty in Reasoning. Unconfirmed diseases are first ruled out by the mandatory questions. When at least one necessary symptom or sign is missing, the disease in question is rejected. If d is a disorder or disease in ONE, and $n(d)$ is the number of questions associated with d in the query data base, the score $S(d)$ of d can be defined as

$$(1) \qquad S(d) = \sum_{i=1}^{n(d)} X_i(d)P_i(d)$$

where $X_i(d)$ is a logical variable equal to 1 if the user of ONE inputs an answer to the nth question; otherwise, $X_i(d)$ is equal to 0; and $P_i(d)$ is the score assigned to (i,d) by the knowledge base according to the answer input to the nth question, such that $P_i(d)$ lies between the minimum and maximum values, $L_i(d)$ and $U_i(d)$, respectively, which are determined by the weight and are also set by the knowledge base. Then

$$(2) \qquad 0 \le S(d) \le \sum_{i=1}^{n(d)} U_i(d)$$

From the result panel it is possible to determine which question ruled out the rejected diseases. After the rejection, three scores are counted for the remaining diseases in order to handle uncertainty. The middle score is the result of computing the scores of all of the answered questions. Some of the questions could have been left unanswered, however. In such cases, ONE counts the answered questions normally, picks the worst alternatives to the unanswered questions, and calculates the lowest of the three scores. For the highest score the best alternatives to the unanswered questions are picked. These three scores are normalized as

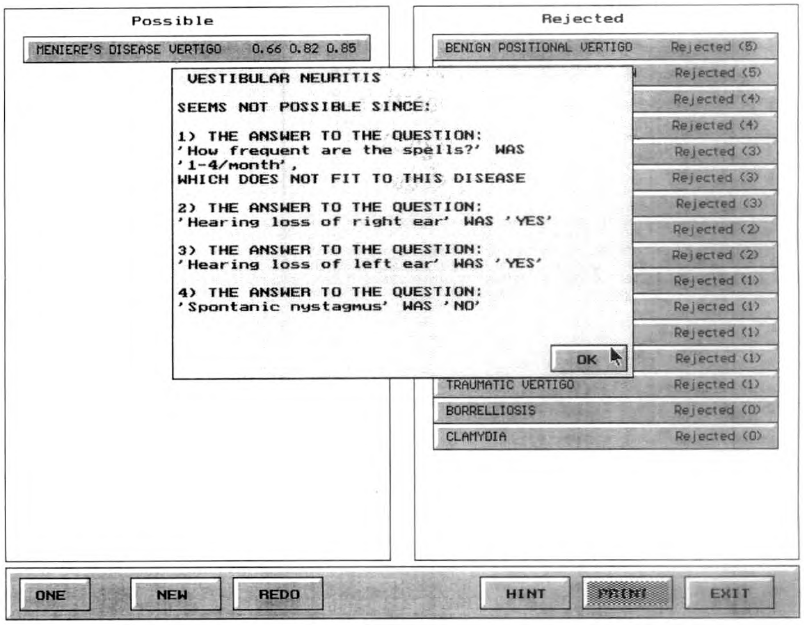
$$l(d) = \frac{\sum_{i=1}^{n(d)} (X_i(d)P_i(d) + (1-X_i(d))L_i(d))}{\sum_{i=1}^{n(d)} U_i(d)}$$

$$(3) \qquad s(d) = \frac{S(d)}{\sum_{i=1}^{n(d)} X_i(d)U_i(d)}$$

$$u(d) = \frac{\sum_{i=1}^{n(d)} (X_i(d)P_i(d) + (1-X_i(d))U_i(d))}{\sum_{i=1}^{n(d)} U_i(d)}$$

where $l(d)$ is the lowest score, $s(d)$ is the main middle score, and $u(d)$ is the highest score. Values $l(d)$, $s(d)$, and $u(d)$ are all less than or equal to 1, according to inequality "2," above (Auramo et al[4]).

If the lowest and highest scores differ greatly, more information (answers) is needed to make the reasoning more certain. The reliability of inference increases as the difference between these two scores becomes narrower. If the highest score is below an earlier-set threshold level, the disease is not consid-



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VESTIBULAR NEURITIS

SEEMS NOT POSSIBLE SINCE:

1) THE ANSWER TO THE QUESTION:
'How frequent are the spells?' WAS
'1-4/month',
WHICH DOES NOT FIT TO THIS DISEASE

2) THE ANSWER TO THE QUESTION:
'Hearing loss of right ear' WAS 'YES'

3) THE ANSWER TO THE QUESTION:
'Hearing loss of left ear' WAS 'YES'

4) THE ANSWER TO THE QUESTION:
'Spontanic nystagmus' WAS 'NO'
```

Fig 3. Result panel with excluded disease.

ered possible.

Result of Reasoning. Once all available information has been entered into the program, reasoning is initiated. The results are presented on one screen. The right-hand side shows the diseases that have been rejected and the number of questions that excluded the disease. The questions can be seen in detail on the screen with the help function if the user is interested (Fig 2). The left-hand side lists the diseases that would seem to be probable (Fig 3). The three scores are presented so that validity of the information can be judged. If the score margin is wide, the program can show what questions should have been answered for the reliability to increase. The disease with the highest score is the most obvious choice, but the other diseases listed on the left-hand side of the screen can also be considered.

Validation. We have the following 18 diseases incorporated into the expert system: 1) acoustic neurinoma, 2) autoimmune disease, 3) benign paroxysmal vertigo of childhood, 4) benign paroxysmal positional vertigo, 5) benign recurrent vertigo, 6) borreliosis, 7) brain stem ischemia, 8) chlamydia, 9) central nervous system tumor, 10) epilepsy, 11) Meniere's disease, 12) neurovascular compression, 13) ototoxicity, 14) perilymphatic fistula, 15) syphilis, 16) traumatic vertigo, 17) vestibular neuritis, and 18) sudden deafness. These diseases include both central and peripheral causes of vertigo. We first tried to select diseases that are common in the general practitioner's office and then completed the list with diseases that offer diagnostic challenges or scientific interest to an otolaryngologist. The goal of validation is to know how well the right diagnosis can be found by using ONE. We are collecting a data base on otolaryngology patients from the University Hospital of Helsinki and intend to compare the expert system's diagnoses to those given by an otolaryngologist. So far, we have run only preliminary tests on 600 cases, but it appears that ONE detects both Meniere's disease and acoustic neurinoma with a high degree of accuracy. These two diseases represent the two large patient groups used by us in the preliminary test trials.

DISCUSSION

With most of the diseases incorporated into ONE the reasoning appears to function well, but we still have some difficulties. Temporal lobe epilepsy can rarely cause vertigo. The problem is not detecting it, but rather excluding it in the reasoning process. The expert system is practical because it can provide differential diagnostic possibilities even with incomplete information. The more information given, the more reliable are the diagnostic choices.

Earlier we also had diagnostic questions in addition to mandatory and supportive questions. The diagnostic questions were removed after the preliminary test, because the reasoning procedure ended if a positive answer to a diagnostic question appeared, and no scores for other diseases were counted. By removing the diagnostic questions, we have tried to increase the amount of information given to the user on all alternative diseases. It also emphasizes that the final diagnostic choice must be made by the user.

Aging of Expert System. One problem with a medical knowledge base is the constantly increasing amount of information. Newly recognized diseases, for example neurovascular compression, for which the diagnostic criteria are still prone to change or increase, offer an additional challenge. The basic reasoning method can remain the same, but the scoring must be evaluated and, if need be, then validated in a set time span in expert systems like ours. The validation is easily done because the scores and weighted values are set independently for every question and disease. In other words, if a new diagnostic test appears for a disease, only the scoring of that test has to be validated.

Reliability of Given Information. For ONE to work optimally, it is essential that the given information be reliable and correct, because the system has to rely on it. The accuracy of reasoning is partly dependent on the user's ability to detect and identify correctly those case facts that are significant to the diagnosis. The presence of two (or more) diseases simultaneously or one major disease earlier may cause difficulties with the reasoning. It is essential that only symptoms associated with the present disease be given. The judging of symptoms is often impossible for the patient and can offer difficulties for the physician, too. Some elderly people can have hearing loss and tinnitus or both as a result of old untreated otitis, chronic noise exposure, or aging, and they can then suddenly experience vertigo with or without these symptoms. It is up to the user of the system to judge which symptoms are associated with the new vertiginous disease. For example, in traumatic vertigo, the beginning of symptoms has to match a head injury.

Necessary questions are used to reject unlikely diseases. We have used, for example, tinnitus to reject certain diseases. While collecting the data base, we were surprised to find how many patients complain of tinnitus. Tinnitus can be vague and present in both ears. Even the patient may have difficulty in localizing it. It is a difficult symptom because it cannot be objectively verified.

Concluding Remarks. We have been able to create an inference program that seems to suit decision-making in a complex medical field. Both patient history and otoneurological test results can be used in the reasoning. The system is useful both as a diagnostic aid and, with the hypertext function, as an educational tool.

REFERENCES

1. Gavilán C, Gallego J, Gavilán J. 'Carrusel': an expert system for vestibular diagnosis. Acta Otolaryngol (Stockh) 1990; 110:161-7.

2. Mira E, Buizza G, Magenes G, Manfrin M, Schmid R. Expert systems as a diagnostic aid in otoneurology. ORL J Otorhinolaryngol Relat Spec 1990;52:96-103.

3. Firriolo FJ, Wang T. Diagnosis of selected pulpal pathoses using an expert computer system. Oral Surg Oral Med Oral Pathol 1993;76:390-6.

4. Auramo Y, Juhola M, Pyykkö I. An otoneurological expert system for the diagnosis of dizziness and vertigo. In: Proceedings of the Finnish Artificial Intelligence Conference, Otaniemi, Finland, 1992:80-9.

5. Auramo Y, Juhola M, Pyykkö I. Otoneurological expert system ONE for vertigo and dizziness. Medical Informatics (in press).

6. Pfaltz CR, Thomsen J. Symptomatology and definition of Meniere's disease. In: Pfaltz CR, ed. Controversial aspects of Meniere's disease. Stuttgart, Federal Republic of Germany: Georg Thieme Verlag, 1986:2-7.

7. Kohut RI. Perilymph fistulas. Clinical criteria. Arch Otolaryngol Head Neck Surg 1992;118:687-92.

8. Calhoun KH, Strunk CL. Perilymph fistula. Arch Otolaryngol Head Neck Surg 1992;118:693-4.

9. Schwaber MK, Hall JW. Cochleovestibular nerve compression syndrome. I. Clinical features and audiovestibular findings. Laryngoscope 1992;102:1020-9.

10. Cummings CW, ed. Otolaryngology–head and neck surgery. 2nd ed. St Louis, Mo: Mosby, 1993.

Ann Otol Rhinol Laryngol 105:1996

HIGH-RESOLUTION COMPUTED TOMOGRAPHIC EVALUATION OF THE COCHLEAR CAPSULE IN OTOSCLEROSIS: RELATIONSHIP BETWEEN DENSITOMETRY AND SENSORINEURAL HEARING LOSS

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Otosclerotic cochlear involvement is a rather frequent disease that has not been clearly understood in terms of diagnosis and management. Objective evaluation methods are needed to confirm the clinical diagnosis, investigate the relationship with hearing impairment, and validate the results of treatment. In this study two ear groups with bone conduction hearing loss (BCHL) were investigated with audiometry and high-resolution computed tomography (HRCT). In the first group (n = 22) the diagnosis of fenestral otosclerosis was confirmed at operation; the second group (n = 9) was composed of ears clinically suspicious for purely cochlear involvement. Additionally, a control group (n = 14) of otologically normal ears was also studied. Foci of demineralization were demonstrated in 58% of the ears in the two groups; the sensorineural hearing loss (SNHL) in those ears was significantly worse than in those with normal radiologic findings. Three methods of HRCT densitometry were used to determine the abnormal regions in the cochlear capsule; the results suggested that hypodense regions were consistent with a greater degree of SNHL, in contrast to the hyperdense ones in ears with better cochlear reserves. Agreement was found between the location of the density change and the frequency topography of the SNHL; densitometric values were correlated with the bone conduction thresholds for certain frequencies. It is concluded that the spongiotic foci are responsible for the SNHL, since there was a correlation between their location and the SNHL frequency. The determination of better hearing in those ears with sclerotic foci supports the hypothesis that the sclerotic phase may not be a healing process following the spongiotic phase, and that it can be the first stage of the disease.

KEY WORDS — densitometry, high-resolution computed tomography, otosclerosis, sensorineural hearing loss.

INTRODUCTION

Otosclerosis is a hereditary disease of the otic capsule in which the mature lamellar bone of the endochondral layer is replaced with more cellular and vascular woven bone. Despite progress in understanding the pathogenesis and advances in the surgical treatment, there are several questions concerning the etiology, biochemistry, and histopathology of the disease.

Otosclerotic cochlear involvement leads to progressive sensorineural hearing loss (SNHL), especially in advanced fenestral disease. In addition, there is a different clinical form, named cochlear otosclerosis, that primarily affects the otic capsule.

The clinical diagnosis of otosclerotic cochlear involvement is established by excluding the other possible causes of SNHL. Similarly, the administration, dosage, and maintenance of sodium fluoride is based on clinical and audiologic data.

High-resolution computed tomography (HRCT) densitometry can be an objective approach, not only for the diagnosis, but also the evaluation of the disease following medical or surgical treatment in order to determine the changes in the extent and maturation of the process.

In this study, the value of HRCT and density measurement methods in the diagnosis of otosclerotic cochlear involvement and the relationships between density change and SNHL are investigated by examining densitometric and audiologic data.

MATERIALS AND METHODS

A total of 25 patients (31 ears) and 7 controls (14 ears) were investigated in the Departments of Otolaryngology and Radiodiagnostics of Dokuz Eylül University Medical School Hospital by audiometry and HRCT. The first group included 22 ears of 20 patients with a mixed hearing loss surgically treated by a small fenestra stapedotomy and Teflon prosthesis technique. The second group was composed of 9 ears of 5 patients with a progressive SNHL that could not be explained by other causes and was regarded as suspicious for cochlear otosclerosis depending on history and clinical and audiologic patterns. The absence of history and/or signs of previous otitis media or other identifiable causes of SNHL such as acoustic trauma, surgical trauma, sudden deafness, endolymphatic hydrops, and age younger than 35 years were also regarded as inclusion criteria. The data of the 14 ears from 7 otologically normal patients were evaluated as controls.

From the Departments of Otolaryngology (E. A. Güneri, Ceryan, A. Güneri) and Radiodiagnostics (Ada), Dokuz Eylül University Medical School, İzmir, Turkey.

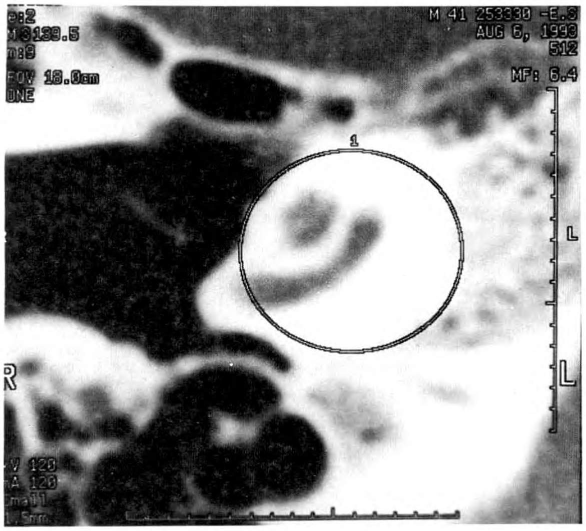

Fig 1. Histogram method.

The air conduction thresholds and bone conduction thresholds (BCTs) of both ears at 0.5, 1, 2, and 4 kHz were examined (AC 5 Digital Audiometry, Interacoustics). Preoperative BCTs were evaluated in the first group; those obtained before the HRCT examination were studied in the second and control groups.

A GE CT/Hi Light Advantage unit (General Electric, Milwaukee, Wis) was used to obtain 1.5-mm-thick nonenhanced axial images using 1.5 mm collimation with a 0.25 mm pixel size and an extended gray scale to 4,000 Hounsfield units (HU; +3,000 to −1,000) for the radiodiagnostic evaluation of the 64 ears in the patient and control groups.

The HRCT evaluation was conducted in two ways: visual evaluation and HRCT densitometry. The densitometric methods were classified into a histogram method and two topographic methods. The topographic methods were modified from those described by Valvassori and Dobben[1] and De Groot and Huizing.[2]

Histogram Method. The mean, maximum, and minimum densities and the frequency of the pixels having a density of 2,000 HU were obtained on an axial cut exposing all cochlear turns (Fig 1). The minimum density was believed not to represent the minimum density of the bony otic capsule and was excluded from the study, since it showed variations due to labyrinthine fluids and air in the middle ear cavity. The 2,000 HU pixel frequency (PF) was regarded as the number of pixels in the area having the normal bony otic capsule density of 2,000 HU.

Densitometric Profile Method. Densitometric values of 15 points on an inferior axial cut exposing the basal coil and round window niche, and 20 points on a superior axial cut passing from the modiolus and exposing all three cochlear coils, were obtained in a manner similar to that described by Valvassori and Dobben.[1] The first 15 points topographically reflected the radiodensity of the basal coil region; points 15 to 25, of the middle coil; and points 25 to 35, of the apical coil (Fig 2).

The densitometric values of the 35 points obtained from the control group were graphically constructed as a normal otic capsule densitometric profile, and the values obtained from the patient group of ears were evaluated accordingly (Fig 3).

Four-Point Densitometry Method. The densitometric values of 6 standard points on an axial cut exposing all cochlear turns were measured as described by De Groot and Huizing[2] (Fig 4). The data of point 5, located medially near the middle ear, and point 6, located laterally near the internal acoustic meatus, were excluded from the study, since the partial vol-

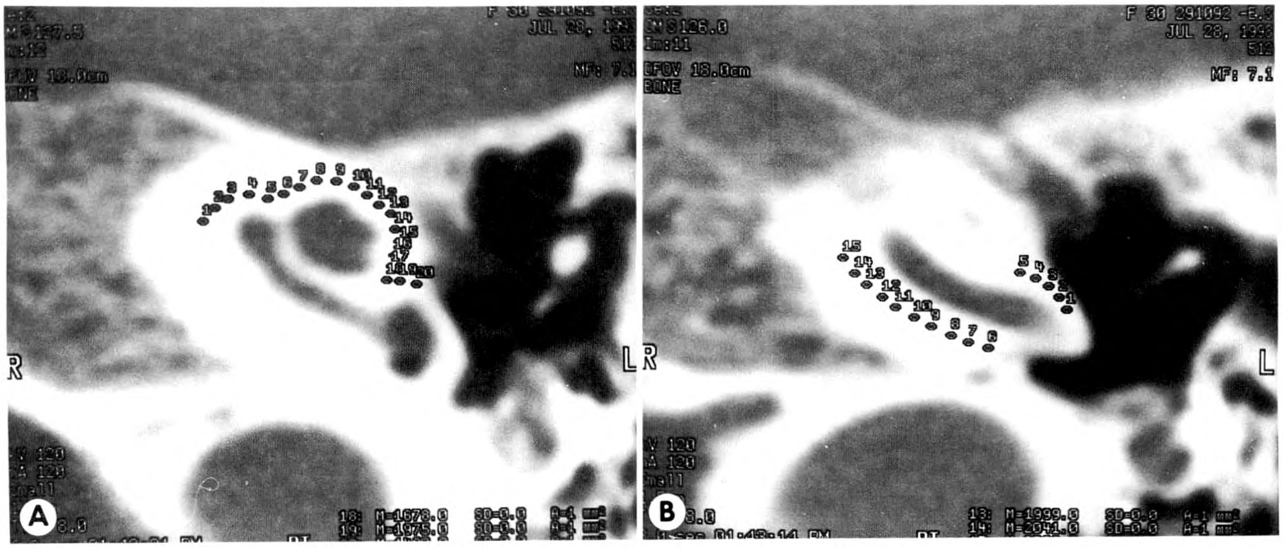

Fig 2. Densitometric profile method. A) Superior cut. B) Inferior cut.

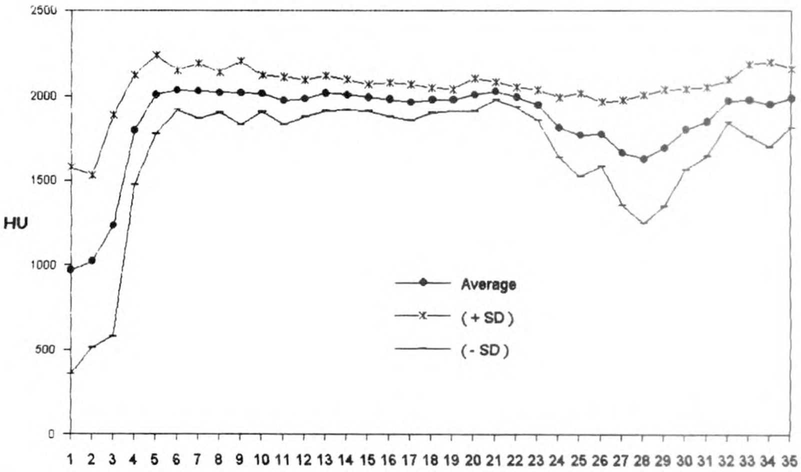

Fig 3. Normal densitometric profile of otic capsule.

ume averaging effects prevented obtaining representative data of the bony otic capsule in those locations.

Student's t-test was used in the statistical analysis of the data; simple linear regression analysis was used to determine the correlation coefficient (R) between each densitometric parameter and the BCTs for different frequencies.

RESULTS

VISUAL EVALUATION

The visual evaluation of the control group scans demonstrated a regular pattern in all cases. In the first patient group, 8 ears (36%) had normal otic capsules, but 14 ears (64%) had radiolucent areas representing demineralization activity. In the second group, 5 ears (55%) had normal otic capsules, and 4 ears (45%) had radiolucent areas.

Since it appeared that the ears with radiolucent areas had elevated thresholds, the BCTs for 0.5, 1, 2, and 4 kHz and the average of the four frequencies were statistically analyzed in order to compare the radiolucent ears with those considered visually normal. In the first group, the differences between the BCTs of visually positive and visually negative ears were not statistically significant for any of the frequencies tested ($p > .05$). The BCT elevations for 1, 2, and 4 kHz and the average of the four frequencies were found to be significant in the visually positive ears of the second group ($p < .05$). When all 31 ears in the two patient groups are considered together, the BCTs for 1, 2, and 4 kHz and the average of the four frequencies were also found to be significantly elevated in those 18 ears that were labeled as visually positive ($p < .05$).

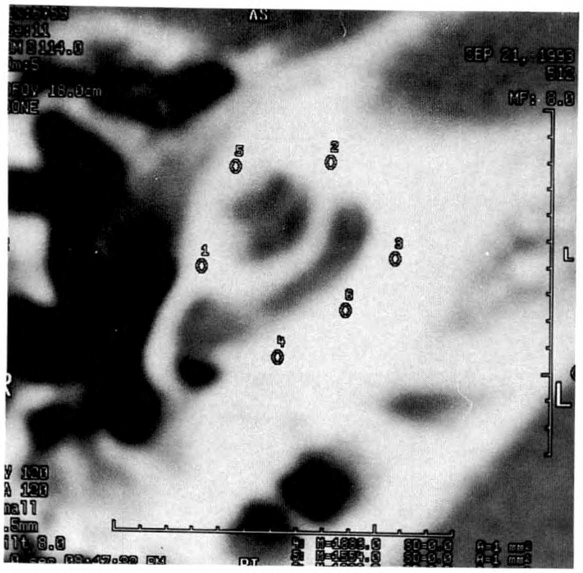

Fig 4. Four-point densitometry method.

HRCT DENSITOMETRY

Histogram Method. The parameters of the histogram method obtained from the control and patient groups are shown in Table 1. The results of the statistical analysis revealed that the mean value was higher in the second group ($p < .05$), the maximum value was higher in both groups ($p < .05$), and the 2,000 HU PF value was lower in both groups than in controls ($p < .05$).

The BCTs of the ears in the second group with a mean value higher than 1,469 HU (control group average + SD) were evaluated and found to be significantly better for 0.5, 1, 2, and 4 kHz and the average of the four frequencies compared with the rest in that group ($p < .05$).

The BCTs of the ears in both groups with a maximum value higher than 2,244 HU (control group average + SD) were evaluated and it was found that they had significantly better thresholds for 2 and 4 kHz and the average of the four frequencies in the first group ($p < .05$), and for all the frequencies tested in the second group ($p < .05$).

TABLE 1. AVERAGE AND STANDARD DEVIATION OF HISTOGRAM METHOD PARAMETERS

	Mean	*Maximum*	*2,000 HU PF*
Controls (n = 14)	1,357 ± 112	2,191 ± 53	84 ± 28
Surgically confirmed otosclerotic ears (n = 22)	1,414 ± 91	2,292 ± 110	50 ± 34
Ears clinically suspicious for otosclerotic cochlear involvement (n = 9)	1,525 ± 157	2,304 ± 107	37 ± 30

HU — Hounsfield unit, PF — pixel frequency.

TABLE 2. AVERAGE AND STANDARD DEVIATION OF FOUR-POINT DENSITOMETRY METHOD PARAMETERS

	Point 1	*Point 2*	*Point 3*	*Point 4*
Controls (n = 14)	1,992 ± 179	1,916 ± 114	1,991 ± 128	1,953 ± 348
Surgically confirmed otosclerotic ears (n = 22)	1,840 ± 339	1,965 ± 230	1,902 ± 262	1,864 ± 460
Ears clinically suspicious for otosclerotic cochlear involvement (n = 9)	2,125 ± 202	2,054 ± 298	2,141 ± 172	2,009 ± 108

When the BCTs of the ears in both groups in which the 2,000 HU PF value was lower than 56 (control group average − SD) were evaluated, it was found that those ears with a lower 2,000 HU PF value did not have a significantly different ($p > .05$) BCT from the rest of the first group; but they had a significantly better ($p < .05$) BCT for all the tested frequencies in the second group.

Densitometric Profile Method. As shown in Fig 3, the normal cochlear capsule radiodensity is 1,000 HU in the anterior basal turn and round window regions, 1,800 HU in the oval window region, and 2,000 HU in the remaining regions. The densitometric values of the first 5 points, located around the round window, and points 25 to 35, located around the oval window, were found to show variations due to the partial volume effect. To obtain normal limits for each point; a value higher than the average control measurement + SD was considered *increased* and a value lower than the average control measurement − SD was considered *decreased.*

In an effort to identify cochlear involvement patterns, the densitometric value of each standard point in all ears of the two patient groups was investigated to find out the frequency and location of abnormal densities. The result showed that none of them had a normal set of 35 points, and there were multiple increased and/or decreased values enabling us to identify hypodense, hyperdense, and mixed involvement patterns occupying the basal, middle, and apical regions or a combination of them.

On the basis of the knowledge that the vibration amplitudes of higher tones are located in the basal turn, and increasing frequency selectivity toward the apex, it seemed that there was a concordance between the frequency of the SNHL and the location of abnormal densitometric points.

Four-Point Densitometry Method. The parameters are shown in Table 2. The results of the statistical analysis revealed that the value of point 1 was significantly lower ($p < .05$) in the first group, and that of point 3 was significantly higher ($p < .05$) in the second group, compared with controls. The differences from the control group were not significant ($p > .05$) for point 2 or point 4 in either group.

The BCTs of the first group ears with a point 1 value lower than 1,813 HU (control group average − SD) were investigated and found to be significantly elevated ($p < .05$) for 1 kHz compared with the rest. When the BCTs of the second group ears with a density of point 3 higher than 2,119 HU (control group average + SD) were evaluated, they were found to be significantly better for 0.5, 1, 2, and 4 kHz and the average of the four frequencies compared with other ears in that group.

The correlations between the densitometric parameters and the BCTs for different frequencies were found to be low in the first group but high in the second group. For the histogram parameters, the highest R was between mean and 2 kHz (.47) in the first group and between maximum and 4 kHz (.88) in the second group. When the four densitometric points were considered, the highest R was between point 1 and 0.5 kHz (.59) in the first group; the strongest correlation in the second group was between point 3 and 2 kHz (.85). The results suggest that each point correlated with the frequency topographically consistent with its location.

DISCUSSION

It is generally believed that the cause of the SNHL is the spiral ligament hyalinization resulting from the toxic effects of the enzymes liberated by the otospongiotic focus[3,4]; the hyalinization has been felt to provide an indication of the amount of toxic enzymes, since there is a positive linear relationship between the amount of hyalinization and the magnitude of the SNHL.[5]

Although both stages of the disease could be found solely or simultaneously in the same focus, absence of hyalinization adjacent to the sclerotic stage lesions suggests that they were sclerotic from the onset and not preceded by spongiotic lesions.[2] This finding questions the long-held belief that maturation of the spongiotic lesions with ossification is the final sclerotic stage of the disease.

The clinical diagnosis of otosclerotic cochlear involvement was formerly confirmed by demonstrating otic capsule demineralization by means of polytomography,[2,6] but modern imaging techniques such as HRCT, and especially densitometry, stimulated a new effort to quantify changes related to the otosclerotic process.

With the higher density resolution brought about by HRCT, otospongiosis affecting the otic capsule can be demonstrated as hypodense bony rarefaction areas ranging from small, isolated foci to diffuse demineralization with complete contour dissolution.[7-9] Generally, a normal layer of cortical bone exists between the pericochlear spongiotic area and the cochlea, giving the classic double-ring appearance.[1,2,6,10,11]

Sclerotic foci are more difficult to demonstrate, since they must have a different density and large size in order to be visualized.[11] It is generally considered that the otic capsule has the maximal bony density and cannot become more dense with the apposition of new bone; a sclerotic lesion can be identified as an area of irregularity or thickness of the capsule.[1,4,9,11]

Surgically confirmed fenestral otosclerosis with a progressive SNHL presumably caused by cochlear involvement may have a normal otic capsule HRCT image. While the cause is the difficulty in discerning the density of the sclerotic foci from the normal otic capsule in most cases, it is possible that some patients may not undergo an otospongiotic demineralization phase.[1,2,4,10]

The reported series of surgically confirmed otosclerotic ears with progressive mixed hearing loss or SNHL indicate a 12% to 100% incidence of HRCT signs consistent with otosclerotic cochlear involvement.[1,2,4,6,12] Swartz et al[4] reported 20 ears with positive HRCT signs of otic capsule involvement in which the frequency of SNHL was correlated with the location of demineralizing foci. In a series of 24 ears, Blakeley et al[10] reported that the average BCTs for 0.5, 1, and 2 kHz of the ears with santral lucencies paralleling the cochlea were not significantly different from the rest.

In this series there were positive HRCT findings in 64% of the ears in the first group, in which the diagnosis was confirmed at the operation. There were positive HRCT signs in 45% of the ears in the second group, which included those ears clinically suspicious for cochlear otosclerosis with progressive SNHL unrelated to other conditions.

It is noteworthy that the densitometric parameters obtained from the ears in the second group indicated higher densities. The mean, maximum, and 2,000 HU PF parameters of the histogram method were all consistent with a significantly hyperdense otic capsule compared with the control and first groups. Similarly, the four-point densitometry method revealed a significantly higher density of point 3 compared with controls. The densitometric profile of the second group showed a predominance of higher density values as well. The relatively low incidence of radiolucencies detected by HRCT was believed to reflect the difficulty in demonstrating the sclerotic phase of the disease solely by visual evaluation. On the other hand, densitometric findings suggested the possibility of an increase in the normal otic capsule density with sclerotic lesions.

The normal otic capsule density is about 2,000 HU and shows little variation; partial volume averaging effects due to the proximity of the middle ear and internal acoustic meatus influence the measurements obtained from thin capsule areas.[1,2,13] The radiodensity of otospongiotic foci demonstrates 20% to 50% attenuation compared with normal cortical bone[2,6,7]; densitometry can quantitatively determine early disease, while visual evaluation can only detect radiologic attenuations greater than 200 HU.[2,13] Densitometry with HRCT has been shown to detect otosclerotic cochlear involvement in 50% of ears with a mixed hearing loss.[2] Valvassori and Dobben[1] reported that HRCT densitometry could demonstrate cochlear involvement in all patients with SNHL in addition to surgically confirmed fenestral disease. Damsma et al[6] and De Groot and Huizing[2] reported that densitometry was able to disclose cochlear involvement in 18% of surgically confirmed otosclerotic ears; they also showed that there was a positive correlation between the location of hypodense foci and hearing perception within the cochlea. While the methodology was different in the two series, both found that the sign of otosclerotic cochlear involvement was radiologic attenuation and that the otic capsule could not become more hyperdense with otosclerotic bone apposition.

The data in this series indicate possibly hyperdense or mixed involvement patterns more likely to be consistent with the histopathologic features of the disease; these findings also indicate that the sclerotic phase can be demonstrated as an increase in the radiologic density in addition to thickness.

CONCLUSIONS

High-resolution computed tomography densitometry should be considered not merely a diagnostic method but also an objective approach for the evaluation of otosclerosis after medical or surgical treatment. Since there is a topographic correlation between the densitometric parameters and BCTs for different frequencies, the results of therapy may be objectively evaluated by considering both densitometric and audiologic data obtained before and after treatment.

REFERENCES

1. Valvassori GE, Dobben GD. CT densitometry of the cochlear capsule in otosclerosis. AJNR 1985;6:661-7.
2. De Groot JAM, Huizing EH. Computed tomography of the petrous bone in otosclerosis and Meniere's disease. Acta Otolaryngol Suppl (Stockh) 1987(suppl 434).
3. Swartz JD, Faerber EN, Wolfson RJ, Marlowe FI. Fenestral otosclerosis: significance of preoperative CT evaluation. Radiology 1984;151:703-7.
4. Swartz JD, Mandell DW, Berman SE, Wolfson RJ, Marlowe FI, Popky GL. Cochlear otosclerosis (otospongiosis): CT analysis with audiometric correlation. Radiology 1985;155: 147-50.
5. El-Rahman AAGA. Cochlear otosclerosis: statistical analysis of relationship of spiral ligament hyalinization to hearing loss. J Laryngol Otol 1990;104:952-5.
6. Damsma H, De Groot JAM, Zonneweld FW, Van Waes PFGM, Huizing EH. CT of cochlear otosclerosis (otospongiosis). Radiol Clin North Am 1984;22:37-43.
7. Jackler RK, Dillon WP. Computed tomography and magnetic resonance imaging of the inner ear. Otolaryngol Head Neck Surg 1988;99:494-504.
8. Milroy CM, Michaels L. Pathology of the otic capsule. J Laryngol Otol 1990;104:83-90.
9. Phelps PD. The basal turn of the cochlea. Br J Radiol 1992;65:370-4.
10. Blakeley BW, Hilger PA, Taylor S, Hilger J. Computed tomography in the diagnosis of cochlear otosclerosis. Otolaryngol Head Neck Surg 1986;94:434-8.
11. Mafee MF, Valvassori GE, Deitch RL, et al. Use of CT in the evaluation of cochlear otosclerosis. Radiology 1985;156:703-8.
12. Huang T-S, Lee F-P, Chen L-K. Radiographically confirmed cochlear otospongiosis among the Chinese. Ann Otol Rhinol Laryngol 1991;100:232-5.
13. Huizing EH, De Groot JAM. Densitometry of the cochlear capsule and correlation between bone density loss and bone conduction hearing loss in otosclerosis. Acta Otolaryngol (Stockh) 1987;103:464-8.

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DIZZINESS AND BALANCE DISORDERS

The Eighth Annual Interdisciplinary Seminar on Diagnostic and Rehabilitative Aspects of Dizziness and Balance Disorders will be held December 4-7, 1996, in Denver, Colorado. For further information, contact Jane Wells, MA, 1996 Balance Meeting, 300 E Hampden Ave, Suite 401, Englewood, CO 80110; telephone (303) 788-4235; fax (303) 788-4234.

Ann Otol Rhinol Laryngol 105:1996

SOFTWARE REVIEW

ADULT AIRWAY MANAGEMENT PRINCIPLES AND TECHNIQUES

Re: Multimedia CD-ROM review, "Adult Airway Management Principles and Techniques," Rafael A. Ortega, MD, and Harold Arkoff, MD, Department of Anesthesiology, Boston University Medical Center.

Company: Silver Platter Education, Inc, 100 River Ridge Dr, Norwood, MA 02062-5043. Telephone (800) 343-0064.

Price: $175.

This interactive educational CD-ROM runs on either a Macintosh or a Windows based personal computer. It requires a CD-ROM player and a soundboard. The authors have put together an excellent CD-ROM incorporating multimedia including excellent photographs, diagrams, audio descriptions, and video clips on topics of airway management. The subjects include sections on anatomy of the airway, management of foreign bodies, and types of endotracheal tubes and laryngoscopes. The techniques are demonstrated for all the basic aspects of placement of endotracheal tubes of various kinds, including laryngeal mask, establishing proper positioning of the endotracheal tube with audio and video and graphical descriptions. It includes a section on syndromes and anomalies that describes the various syndromes and how they may be difficult from the point of view of establishing an airway; however, no use is made of video in this section, and many of the syndromes did not have a picture of an involved individual. The installation was very straightforward and it ran very well on a Windows based system. There is a video browser included so that the user can jump right to videos covering how to mask-ventilate, use of a pulse oximeter, spirometry, suctioning, etc.

The user is presented initially with a list of topics, and each topic is covered in pages with the ability to access a notepad or view the text of the audio description. Every page in every chapter is annotated by audio description by a professional annotator. Navigating through the system is self-evident because of arrows and text-labeled buttons. The user can take an examination for continuing medical education credits. It is possible to capture or print individual screens. It is also possible to zoom a video or diagram to view details in the larger format according to the capabilities of one's system. An appendix is included with references that can be printed.

The topics are certainly relevant to otolaryngologists in terms of the basic techniques for intubation, management of the airway in patients under any type of general anesthetic, and an understanding of the monitoring equipment that is available in the operating room. This should be a required CD-ROM for beginning residents in otolaryngology–head and neck surgery. It is presented with very appealing, sophisticated, relevant graphics and is very easy to use. It is also approved for 4 Category 1 credit hours of the Physician's Recognition Award of the American Medical Association. I give this CD-ROM a very high rating.

KEITH F. CLARK, MD

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IMAGING CASE STUDY OF THE MONTH

METASTATIC BREAST CARCINOMA PRESENTING AS CAVERNOUS SINUS SYNDROME

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CHRISTOPHER H. RASSEKH, MD GREGORY CHALJUB, MD
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INTRODUCTION

Patients with cranial nerve palsies caused by skull base disease can present a diagnostic challenge. The cavernous sinus in particular may be affected by a variety of pathologic processes. Computed tomography (CT) and magnetic resonance imaging (MRI) greatly improve our ability to diagnose these conditions, but skull base imaging alone is not always diagnostic, and other clues from the history and physical examination may be needed to make the diagnosis. We report a case in which skull base imaging helped make the diagnosis of metastatic breast carcinoma in a patient presenting with the cavernous sinus syndrome.

CASE REPORT

A 49-year-old woman was referred to the University of Texas Medical Branch Otolaryngology Clinic complaining of a 6-month history of diplopia and recurrent right retrobulbar headaches. At an outside hospital the patient had undergone CT and MRI that showed a skull base mass of the upper and middle part of the clivus consistent with chordoma. The patient's past medical history was unremarkable. The physical examination revealed right-sided cranial nerve deficits: mild ptosis, abducens paralysis, a decreased corneal reflex, and decreased sensation in the distribution of the first and second divisions of the trigeminal nerve. The findings on head and neck examination (including visual acuity) and neurologic examination were otherwise unremarkable.

The CT with contrast performed at our hospital showed a partially enhancing mass within the middle to upper clivus, invading the right cavernous sinus and destroying large areas of bone (Fig 1). The MRI showed a heterogeneous enhancing mass (with use of gadopentetate dimeglumine contrast) with its epicenter in the clivus, extending into the right cavernous sinus and pituitary fossa (Fig 2). The right cavernous carotid was encased and compressed (Fig 2). The lesion abutted the left cavernous carotid and the optic chiasm, but did not impinge on these structures.

In this case, careful review of systems by the otolaryngologist raised the suspicion of breast cancer, and the patient was referred to the gynecology clinic. Examination there revealed a 3.5 × 3.5-cm right retro-areolar breast mass. Fine needle aspiration of the mass was positive for adenocarcinoma. Neurosurgery consultation was obtained, and the cranial base team decided that surgery, including needle biopsy of the skull base mass, would not be performed because of the poor prognosis. The patient was started on steroids and referred to the medical oncology and radiation oncology departments, but died before any other treatment could be instituted. Permission for autopsy was denied. Although pathologic confirmation of the skull base lesion was not obtained, we believe the skull base mass represented metastatic breast carcinoma.

DISCUSSION

Cavernous sinus syndrome (also called parasellar syndrome or painful ophthalmoplegia) is caused by disruption of the cranial nerves of the parasellar region. Patients typically present with facial pain or headaches and paresis of cranial nerves III through VI.[1] The syndrome may be caused by a variety of neoplastic, infectious, inflammatory, or vascular conditions.[1-6] The mode of symptom onset and pattern of neurologic deficit are generally not helpful in determining the cause.[2] Therefore, CT and MRI are often required to make the correct diagnosis.

The CT and MRI findings of our patient were consistent with chordoma, chondrosarcoma, or meta-

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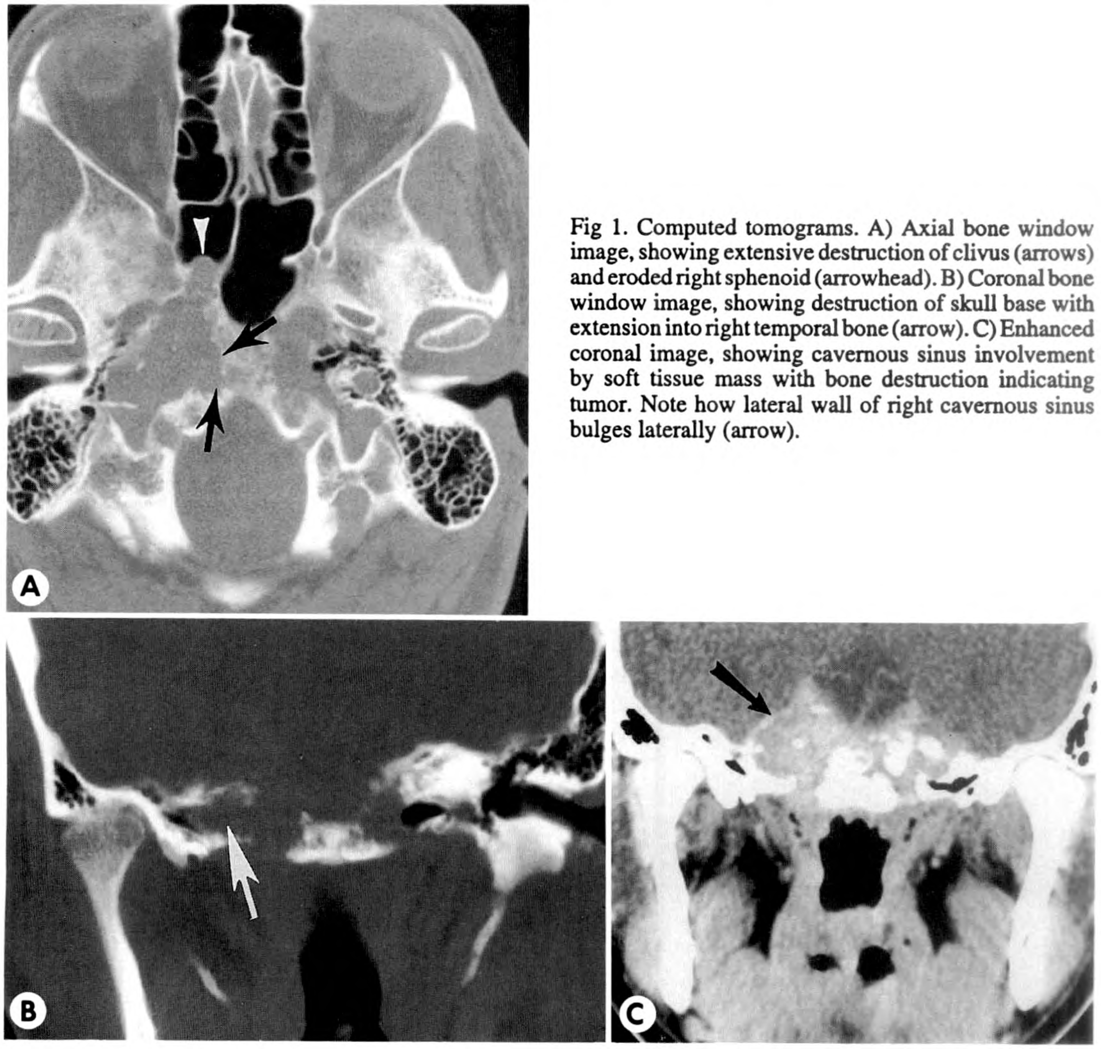

Fig 1. Computed tomograms. A) Axial bone window image, showing extensive destruction of clivus (arrows) and eroded right sphenoid (arrowhead). B) Coronal bone window image, showing destruction of skull base with extension into right temporal bone (arrow). C) Enhanced coronal image, showing cavernous sinus involvement by soft tissue mass with bone destruction indicating tumor. Note how lateral wall of right cavernous sinus bulges laterally (arrow).

static tumor. Chordoma (which accounts for less than 1% of all intracranial tumors[7]) appears as an enhancing soft tissue mass with bone destruction and commonly contains calcifications or fragments of eroded bone.[8] The skull base is involved in 35% of cases of chordoma, and about half of these involve the clivus.[9,10] Chondrosarcoma (which accounts for less than 0.16% of all intracranial tumors[11]) is difficult to distinguish radiologically from chordoma. The tumor appears as a heterogeneously or partially enhancing soft tissue mass with a polymorphous calcified matrix that has been described as flecks, clumps, rings, or eggshells.[10,12-14] Frequently, erosion or destruction of bone without a periosteal reaction is seen, and a sharp zone of transition to normal tissue can be discerned.[10] In a series with 16 cases of chordoma and 9 cases of chondrosarcoma in the clivus, Oot et al[15] found no imaging features to distinguish the two by CT or MRI.

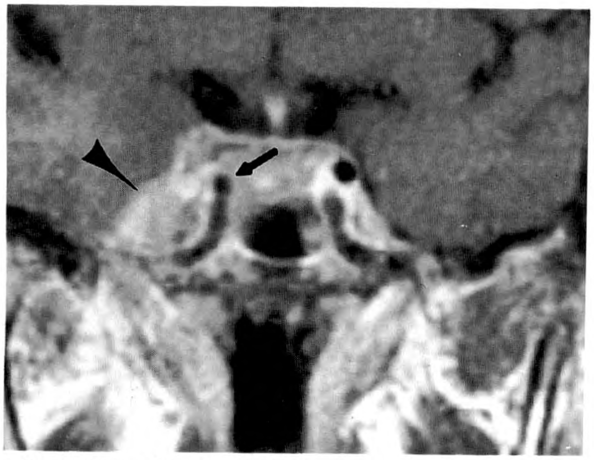

Fig 2. Coronal post-gadolinium magnetic resonance image showing right cavernous carotid artery encasement by tumor (arrow) and abnormally convex lateral wall (arrowhead).

Hematogenous metastases to the skull base are more prevalent than primary tumors,[16] and breast cancer is the most common source.[2,10,17] Other common metastases to the skull base are from lung, kidney, and prostate cancers. All are seen as lytic bone destruction on CT, with or without a soft tissue mass, except for metastases of prostate cancer, which tend to cause an osteoblastic reaction.[16] Metastatic tumors appear hyperintense relative to adjacent bone marrow on T2-weighted MRI. T1-weighted images may show replacement of normal bone marrow by material of decreased signal intensity.[10] Newer imaging techniques such as short tau inversion-recovery (STIR), fast spin-echo, and inversion-recovery fast spin-echo have been described in imaging vertebral metastases,[18,19] bone tumors,[20] and other bone marrow disorders[21] and may be expected to improve the diagnostic power of skull base imaging in the near future.

In 1970 Thomas and Yoss[2] published a series of 102 patients with "parasellar syndrome." They reported 8 cases that were due to metastatic breast carcinoma. In another series,[1] breast carcinoma was the most common cancer to metastasize to the skull base. Seventeen of 43 patients with skull base metastasis had breast cancer; 2 of these patients developed cavernous sinus syndrome. Other common primary sites in both series were lung, head and neck, kidney, and prostate. Hall et al[22] reviewed the records of 7,725 breast cancer patients treated at The M. D. Anderson Hospital from January 1971 to April 1978. They reported 8 cases of cranial nerve palsies due to skull base metastasis. Three of these patients had palsies of cranial nerves III and/or VI. We were unable to identify in the literature any prior case in which cavernous sinus syndrome was the presenting feature of undiagnosed breast cancer.

The cavernous sinus syndrome may be caused by a variety of pathologic processes. Unfortunately, the history and neurologic examination are frequently not helpful in differentiating among them. Both CT and MRI are essential in narrowing the differential diagnosis and defining the extent of the lesion, but they are not always diagnostic. In our case, CT was better at demonstrating cortical bone destruction, but MRI was better at defining the full intracranial extent of tumor. This is consistent with previous reports by other authors.[9,23] While newer imaging sequences may soon improve the diagnostic capability of MRI of the skull base, our case illustrates that a thorough history including a review of systems and a thorough physical examination are required to make a correct diagnosis and avoid unnecessary surgical procedures.

REFERENCES

1. Greenberg HS, Deck MD, Vikram B, Chu FCH, Posner JB. Metastasis to the base of skull: clinical findings in 43 patients. Neurology 1981;31:530-7.
2. Thomas JE, Yoss RE. The parasellar syndrome: problems in determining etiology. Mayo Clin Proc 1970;45:617-23.
3. Bumpous JM, Maves MD, Gomez SM, Levy BK, Johnson F. Cavernous sinus involvement in head and neck cancer. Head Neck 1993;15:62-6.
4. Julien J, Ferrer X, Droullard J, Philippe JC, Desbordes P. Cavernous sinus syndrome due to lymphoma. J Neurol Neurosurg Psychiatry 1984;47:558-60.
5. Rucker CW. The causes of paralysis of the third, fourth and sixth cranial nerves. Am J Ophthalmol 1966;61:1293-7.
6. Zahra M, Tewfik HH, McCabe BF. Metastases to the cavernous sinus from primary carcinoma of the larynx. J Surg Oncol 1986;31:69-70.
7. Kendall BE, Lee BCP. Cranial chordomas. Br J Radiol 1977;50:687-98.
8. Heffelfinger MJ, Dahlin DC, MacCarty CS, Beabout JW. Chordomas and cartilaginous tumors at the skull base. Cancer 1973;32:410-20.
9. Laine FJ, Nadel L, Braun IF. CT and MR imaging of the central skull base. Part 2. Pathologic spectrum. Radiographics 1990;10:797-821.
10. Ginsberg LE. Neoplastic diseases affecting the central skull base: CT and MR imaging. AJR Am J Roentgenol 1992; 159:581-9.
11. Berkmen YM, Blatt ES. Cranial and intracranial cartilaginous tumours. Clin Radiol 1968;19:327-33.
12. Grossman RI, Davis KR. Cranial computed tomographic appearance of chondrosarcoma of the base of the skull. Radiology 1981;141:403-8.
13. Lee YY, Van Tassel P. Craniofacial chondrosarcomas: imaging findings in 15 untreated cases. AJNR Am J Neuroradiol 1989;10:165-70.
14. Bahr AL, Gayler BW. Cranial chondrosarcomas. Radiology 1977;124:151-6.
15. Oot RF, Melville GE, New PF, et al. The role of MR and CT in evaluating clival chordomas and chondrosarcomas. AJNR Am J Neuroradiol 1988;9:715-23.
16. Whelan MA, Reede DL, Meisler W, Bergeron RT. CT of the base of the skull. Radiol Clin North Am 1984;22:177-217.
17. Roessmann U, Kaufman B, Friede RL. Metastatic lesions in the sella turcica and pituitary gland. Cancer 1970;25:478-80.
18. Jones KM, Schwartz RB, Mantello MT, et al. Fast spin-echo MR in the detection of vertebral metastases: comparison of three sequences. AJNR Am J Neuroradiol 1994;15:401-7.
19. Mehta RC, Marks MP, Hinks RS, Glover GH, Enzmann DR. MR evaluation of vertebral metastases: T1-weighted, short-inversion-time inversion recovery, fast spin-echo, and inversion-recovery fast spin-echo sequences. AJNR Am J Neuroradiol 1995;16:281-8.
20. Golfieri R, Baddely H, Pringle JS, et al. Primary bone tumors. MR morphologic appearance correlated with pathologic examinations. Acta Radiol 1991;32:290-8.
21. Jones KM, Unger EC, Granstrom P, Seeger JF, Carmody RF, Yoshino M. Bone marrow imaging using STIR at 0.5 and 1.5 T. Magn Reson Imaging 1992;10:169-76.
22. Hall SM, Buzdar AU, Blumenschein GR. Cranial nerve palsies in metastatic breast cancer due to osseous metastasis without intracranial involvement. Cancer 1983;52:180-4.
23. Sze G, Uichanco LS, Brant-Zawadski MN, et al. Chordomas: MR imaging. Radiology 1988;166:187-91.

IMAGING CASE STUDY OF THE MONTH
HYPOPHARYNGEAL DILATATION IN A MUSICIAN

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INTRODUCTION

Hypopharyngeal dilatation in musicians is not uncommon, but it is usually mentioned secondarily to laryngoceles as sequelae from increased pharyngeal pressure. A marked dilatation of the hypopharynx is not to be confused with a hypopharyngeal pharyngocele, although both are thought to result from forceful exhalation or inflation of the pharynx.[1,2] Here is presented a case of marked hypopharyngeal dilatation in a trumpet player, along with its conservative management.

CASE REPORT

A 19-year-old male trumpet player reported a several-year history of intermittent anterior neck swelling. This neck swelling, which occurred only while he was playing the trumpet, had increased in severity over the preceding year and was now accompanied by cervical pain and transient dysphagia to solids.

Symmetric anterolateral neck swelling without crepitus was observed when he played a trumpet that he had brought with him to clinic. Identical findings were readily demonstrated on physical examination during a modified Valsalva's maneuver. Flexible fiberoptic transnasal pharyngolaryngoscopy demonstrated a pharyngeal mucosa that was normal-appearing but had an abnormally capacious inflatability.

Anterior and lateral cervical films were obtained, both with the patient in repose (Fig 1) and with him performing a modified Valsalva's maneuver (Fig 2). A markedly distensible air-filled pharynx was noted, with no extravasation of air into the soft tissues. There was no evidence of a laryngocele.

The patient had no evidence, either clinically or by history, of a connective tissue disorder. He was advised to 1) seek professional instruction in order to improve his technique, 2) strengthen his neck through exercise, and 3) carefully apply an Ace bandage around his neck for external cervical compression during trumpet playing.

He followed these suggestions, with the exception

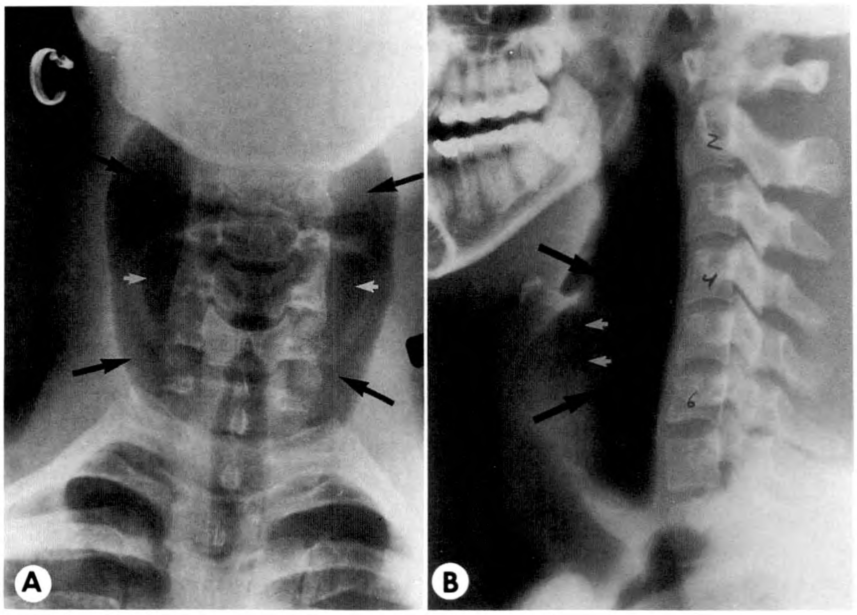

Fig 1. Radiographs of neck with patient at rest. There is no indication of anatomic abnormality. A) Anterior. B) Lateral.

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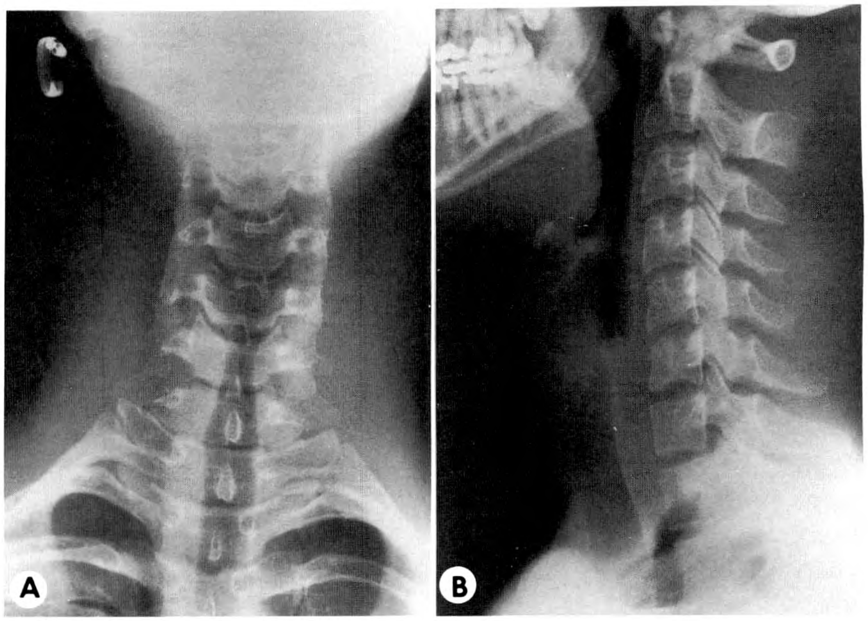

Fig 2. Radiographs of neck taken while patient performed modified Valsalva's maneuver. Symmetric distensibility of hypopharynx is illustrated. Arrows — area of inflated hypopharynx, arrowheads — distended pyriform sinuses. A) Anterior. B) Lateral.

that he substituted a folded bandanna for the Ace bandage around his neck. On follow-up examination, 2 and 6 months later, he noted diminution in both neck bulging and pain with trumpet playing. He has subsequently relearned correct breathing and embouchure under the tutelage of a professional concert musician. Since adopting the corrected techniques, he has had no further problems.

DISCUSSION

The pressures necessary for playing wind instruments can be quite high. In playing the trumpet, mouthpiece pressures greater than 100 psi are frequently exceeded as pitch and intensity are increased.[3] With their better techniques, however, skilled trumpeters can play with much less force than those less proficient.[3]

It is not unusual that some neck expansion occurs during trumpet playing.[1] Consensus among professional wind instrument players, however, is that this expansion is usually asymptomatic.[1]

Treatment of this patient initially involved careful wrapping of the neck before instrument playing. Wrapping can provide external compression against which the increased pharyngeal pressures are braced. Neck muscle strengthening exercises are also felt to structurally stabilize the pharyngeal column, thus reducing the likelihood for lateral dilatation.

The patient was also referred for a professional review of his breathing and trumpet-playing techniques. Professional instructors can make recommendations and work with the musician to reduce the playing force, correct breathing problems, and improve overall playing proficiency. If these efforts fail, playing force may still be reduced by enlarging the mouthpiece, which may require a change to a different musical instrument.

REFERENCES

1. Smith R, Levine H. Hypopharyngeal dilatation in musicians. Med Problems Performing Artists 1986;1:20-3.

2. Norris CW. Pharyngoceles of the hypopharynx. Laryngoscope 1979;89:1788-807.

3. Barbenel JC, Kenny P, Davies JB. Mouthpiece forces produced while playing the trumpet. J Biomech 1988;21:417-24.

Ann Otol Rhinol Laryngol 105:1996

PATHOLOGY CONSULTATION

PHARYNGEAL HYPOPHYSIS

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Derived from embryologic remnants of Rathke's pouch and its craniopharyngeal duct, the pharyngeal hypophysis has long been considered to be present in all humans. This thesis has been recently challenged, but the weight of investigative evidence still favors the original premise. The pharyngeal hypophysis is just one product of Rathke's pouch. Others are extracranial pituitary tumors, the very rare craniopharyngioma, pharyngeal hypopharyngeal cysts, and cerebrospinal fluid rhinorrhea.

The anterior pituitary gland (adenohypophysis) derives from the ectoderm of the stomodeum as a pseudo-invagination caused by proliferation of surrounding mesenchyme and the folding of the embryo during the third and fourth weeks of gestation.[1] This rostral outpouching of the primitive oral cavity, called Rathke's pouch, passes up through the future site of the body of the sphenoid bone (via the craniopharyngeal duct) to the sella turcica, where it combines with the infundibular process of the neural tube, giving rise to the anterior lobe, pars tuberalis, and pars intermedia of the pituitary gland.[2] Obliteration of Rathke's pouch and the craniopharyngeal duct is said to be complete by the 12th week of gestation.[3]

PHARYNGEAL HYPOPHYSIS

The thoroughness of the obliteration is, however, questioned by the findings of McGrath.[4-7] In extensive studies, she has shown that a functioning pharyngeal hypophysis is found in everyone. Furthermore, she has demonstrated a size-volume variability of the extracranial hypophysis with sex and age and also that every hormone elaborated by the sellar hypophysis is found in the pharyngeal gland.

The human pharyngeal hypophysis is an elongated body in the mucoperiosteum of the roof of the nasopharynx, with its long axis lying in the midsagittal plane (Fig 1[4-6]). An anterior oblique part passes deeply and posteriorly from the epithelial surface, through the seromucous gland layer, into a larger horizontal part that extends posteriorly in the periosteum covering the body of the sphenoid's inferior surface. After a survey of 133 so-located hypophyses, McGrath[4,5] gave the following maximum dimensions of the pharyngeal hypophysis: length 9.6 mm, width 1.5 mm, depth 1.0 mm, and volume 1.2 mm^3.

Histologically, the pharyngeal hypophysis has a poorly defined capsule and a cellular constituency (chromophobe, acidophil, basophil) in approximately the same proportions as the sella's anterior pituitary.[4] In necropsy studies, the site of the pharyngeal hypophysis can be represented only by collections of squamous cells.[2]

If the pharyngeal hypophysis is in all of us, why is it that surgical pathologists do not find it in adenoidectomy specimens? Richards and Evans[8] tentatively concluded that routine adenoidectomy does not encompass the hypophysis, but that so-called radical adenoidectomy is capable of so doing.

CRANIOPHARYNGEAL CANAL

An occasionally seen (magnetic resonance and computed tomography scan images) bony canal in the sphenoid, called either the craniopharyngeal canal or the intrasphenoidal canal, has been suggested as the route of the craniopharyngeal duct from the pharynx to the sella turcica.[1,9] More than that, its presence serves as a basis to deny much of McGrath's work.

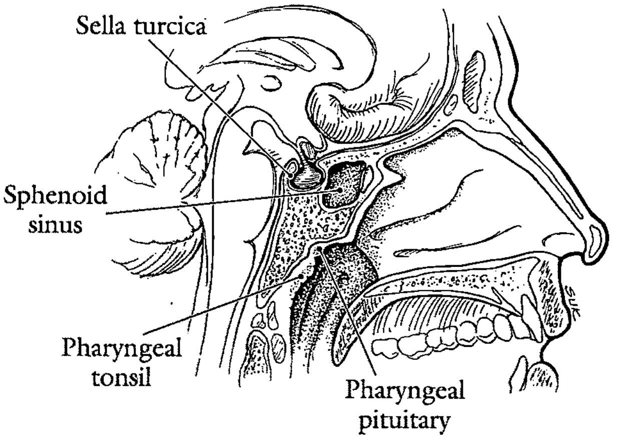

Fig 1. Schematic showing location of pharyngeal hypophysis in humans (as described by McGrath[4-6]).

From the Department of Pathology, The University of Texas M. D. Anderson Cancer Center, Houston, Texas.

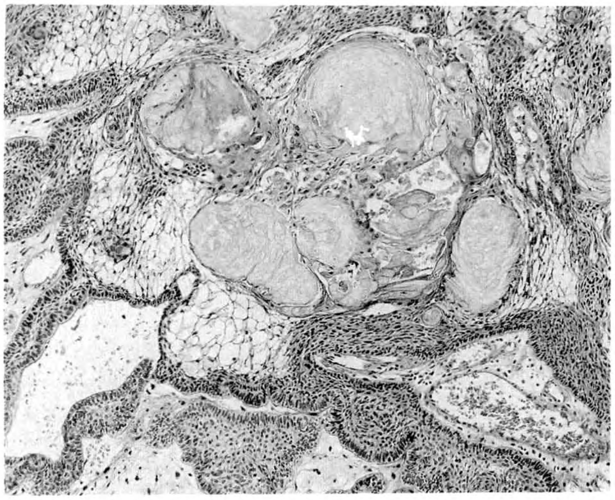

Fig 2. Intracranial craniopharyngioma with its ameloblastoma-like structure and hyalinized extracellular masses (H & E, original ×120).

In normal perinatal development of the cranial base, the sphenoid synchondrosis and the sphenobasilar synchondrosis are active growth sites.[9] The two synchondroses are oriented in the same direction as the intersphenoidal canal, and when the latter is present, it is located in the postsphenoid bone between the two synchondroses.[9] The canal seemingly connects the cerebral cranial base region with the pharyngeal cranial base region.

According to Kjaer and Fischer-Hansen,[1] the canal is a pathologic finding and its presence indicates the presence of pharyngeal pituitary tissue. These investigators found neither pharyngeal pituitary tissue nor canals in normal fetuses.[1,9] They also postulate that pharyngeal pituitary tissues may be implicated in various degrees of craniofacial malformations in the region where interactions among ectoderm of the embryonic pharynx (adenohypophysis), neuroectoderm (neurohypophysis), and mesoderm (sella turcica) take place.

DISORDERS OF EXTRACRANIAL RATHKE'S POUCH AND RELATED STRUCTURES

Disorders related to the pharyngeal hypophysis, remnants of Rathke's pouch or duct, and the intrasphenoidal canal are rare and may be classified into three categories: 1) neoplasms, 2) cysts, and 3) patency of the canal.

Extracranial pituitary adenomas have already been presented in this Pathology Consultation series[10]; craniopharyngiomas have not. Craniopharyngiomas comprise about 3% of all intracranial tumors and are usually found below the brain and above the pituitary, behind the optic chiasm and within the circle of Willis. Intrasellar craniopharyngiomas are quite unusual, and an intrasphenoidal or pharyngeal location, unrelated to downward extension of an intracranial tumor, is exceedingly rare (Fig 2). Lewin et al[2] critically reviewed earlier reports of craniopharyngiomas in the nasopharynx and accepted only one as primary at that site.

The incidence of cysts in the pharyngeal hypophysis is considerably lower than that of cysts in the sellar hypophysis. Like nearly all Rathke's cleft cysts, those in the pharynx are asymptomatic.[6,11]

Cerebrospinal fluid rhinorrhea can occur with a persistent patency of the intrasphenoidal canal.[3]

REFERENCES

1. Kjaer I, Fischer-Hansen B. The adenohypophysis and the cranial base in early human development. J Craniofac Genet Dev Biol 1995;15:157-61.
2. Lewin R, Ruffolo E, Saraceno C. Craniopharyngioma arising in the pharyngeal hypophysis. South Med J 1984;77:1519-23.
3. Herzog J, Makek M, Fisch U. Lesions of Rathke's duct: another indication for the infratemporal fossa approach. Otolaryngol Head Neck Surg 1989;101:302-8.
4. McGrath P. Volume and histology of the human pharyngeal hypophysis. Aust NZ J Surg 1967;37:16-27.
5. McGrath P. The volume of the human pharyngeal hypophysis in relation to age and sex. J Anat 1971;110:275-82.
6. McGrath P. Cysts of sellar and pharyngeal hypophyses. Pathology 1971;3:123-31.
7. McGrath P. Aspects of the human pharyngeal hypophysis in normal and anencephalic fetuses and neonates and their possible significance in the mechanism of its control. J Anat 1978;127:65-81.
8. Richards SH, Evans ITG. The pharyngeal hypophysis and its surgical significance. J Laryngol Otol 1974;88:937-46.
9. Kjaer I, Russell BG. The craniopharyngeal canal indicating the presence of pharyngeal adenopituitary tissue. Eur J Radiol 1995;20:212-4.
10. Langford L, Batsakis JG. Pituitary gland involvement of the sinonasal tract. Ann Otol Rhinol Laryngol 1995;104:167-9.
11. Rottenberg GT, Chong WK, Powell M, Kendall BE. Cyst formation of the craniopharyngeal duct. Clin Radiol 1994;49:126-9.

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VOLUME 105 (9) — SEPTEMBER 1996

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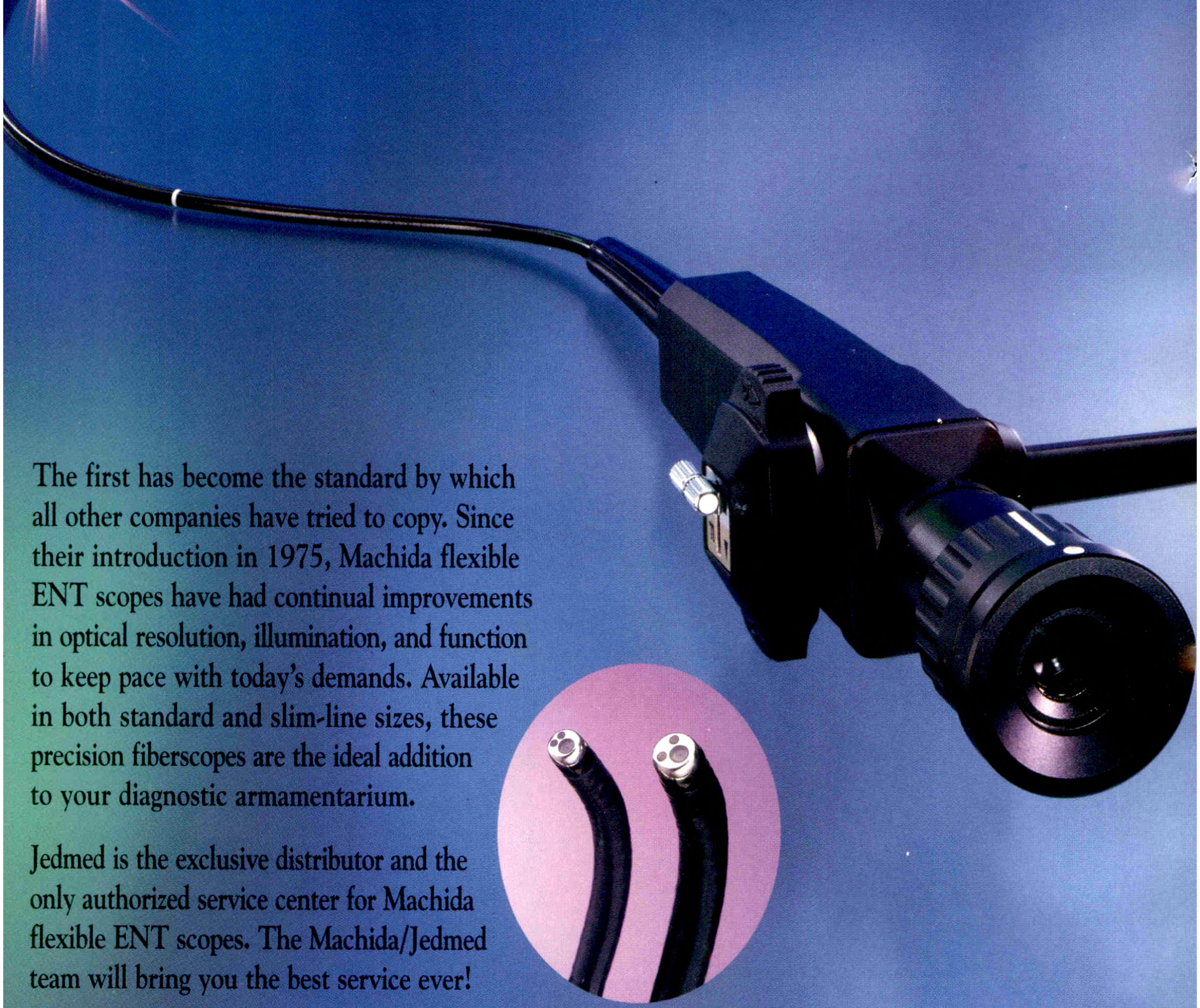

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ANNALS OF OTOLOGY, RHINOLOGY & LARYNGOLOGY

VOLUME 105 SEPTEMBER 1996 NUMBER 9

CONTENTS

INDEPENDENT CONTRIBUTIONS

Ultrastructural Study of Peripheral Nerve Injury Induced by Monopolar and Bipolar Diathermy
Yuval Zohar, MD, DDS; Rima Sadov, MD;
Michael Strauss, MD; Meir Djialdetti, MD 673

Sleep Fluoroscopy for Localization of Upper Airway Obstruction in Children
Sharon E. Gibson, MD; Charles M. Myer III, MD;
Janet L. Strife, MD; David M. O'Connor 678

Meningitis After Adenoidectomy: An Anatomic Explanation
Glenn Isaacson, MD; Wesley W. Parke, PhD 684

Electrical Pacing of the Paralyzed Human Larynx
David L. Zealear, PhD; Cheryl L. Rainey, MS; James L. Netterville, MD;
Garrett D. Herzon, MD; Robert H. Ossoff, DMD, MD 689

Role of the Agger Nasi Cell in Chronic Frontal Sinusitis
Eugenie Brunner, MD; Joseph B. Jacobs, MD; Richard A. Lebowitz, MD;
Barry A. Shpizner, MD; Roy A. Holliday, MD 694

Computer-Aided Three-Dimensional Reconstruction in Human Cochlear Maps: Measurement of the Lengths of Organ of Corti, Outer Wall, Inner Wall, and Rosenthal's Canal
Atsushi Kawano, MD; H. Lee Seldon, MD; Graeme M. Clark, FRACS 701

Plasma Platelet-Derived Growth Factor: Preliminary Study of a Potential Marker in Head and Neck Cancer
Lyon L. Gleich, MD; Laxmi Srivastava, DVM, PhD; Jack L. Gluckman, MD 710

Nuclear DNA Content of the Tall Cell Variant of Papillary Carcinoma of the Thyroid Gland
Yoram Stern, MD; Ora Medelia, PhD; Meora Feinmesser, MD;
Thomas Shpitzer, MD; Raphael Feinmesser, MD; Karl Segal, MD 713

Kinematic and Dynamic Characteristics of Solid Pellet Movement During the Pharyngeal Phase of Swallowing
Mark K. Kern, MS; Junlong Ren, MD; Ronald C. Arndorfer;
Candy L. Hofmann; Reza Shaker, MD 716

Cause of Eustachian Tube Constriction During Swallowing in Patients With Otitis Media With Effusion
Haruo Takahashi, MD; Makoto Miura, MD;
Iwao Honjo, MD; Akihiko Fujita, MD 724

Speech Results and Complications of Near-Total Laryngectomy
Javier Gavilán, MD; Jesús Herranz, MD;
Pilar Prim, MD; Iñaki Rabanal, MD 729

Recordings of Mucociliary Activity In Vivo: Benefit of Fast Fourier Transformation of the Photoelectric Signal
Sven Lindberg, MD, PhD; Anders Cervin, MD, PhD;
Thomas Runer, MD, PhD; Lars Thomasson, MScEE 734

Historical Vignette: Sir William Hingston, Nasal Fibromas, and the Upside-Down Operation
Jack Cohen, MD 746

IMAGING CASE STUDY OF THE MONTH

Schwannoma of the Brachial Plexus
Peter Zbären, MD; Minerva Becker, MD 748

CLINICOPATHOLOGICAL CONSULTATION

Hodgkin's Disease: Old and Recent Clinical Concepts
Antonino Carbone, MD; Lawrence M. Weiss, MD;
Annunziata Gloghini, PhD; Alfio Ferlito, MD 751

MISCELLANEOUS

Information for Subscribers 2a

Index to Advertisers 16a

Ann Otol Rhinol Laryngol 105:1996

ULTRASTRUCTURAL STUDY OF PERIPHERAL NERVE INJURY INDUCED BY MONOPOLAR AND BIPOLAR DIATHERMY

YUVAL ZOHAR, MD, DDS
MICHAEL STRAUSS, MD
RIMA SADOV, MD
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The effect of unipolar and bipolar electrocoagulation on human and albino rat peripheral nerves was examined by transmission electron microscopy. The greater auricular nerve obtained from operated patients and the femoral nerve of an albino rat were chosen for this study. The nerves were divided into three groups: controls, segments of nerves spot-coagulated with unipolar instruments, and segments of nerves spot-coagulated with bipolar instruments. Significant cell injury, expressed by marked damage of the Schwann cells, was induced by unipolar coagulation in both myelinated and unmyelinated nerves, in humans as well as in the albino rat. However, in bipolar-treated nerves, the Schwann cell architecture remained well preserved in both types of nerves. The results are in favor of bipolar electrocoagulation when applied in areas rich in neurovascular supplies.

KEY WORDS — bipolar coagulation, electrocoagulation, electron microscopy, peripheral nerve, unipolar coagulation.

INTRODUCTION

From surgery's beginnings and well into the 19th century, a cautery heated over a bed of hot coals was regularly applied to control hemorrhage. Rapid coagulation, resulting in decreased bleeding, increases visualization of the field and shortens operating time. It is known, however, that cautery injury to cranial nerves and their branches remains an intraoperative risk in head and neck surgery. Diathermy coagulation may destroy neural tissue adjacent to and beyond the intended site.

Although electrocoagulation has long been used for control of operative bleeding, electron microscopic examination of the neural injuries has not been reported, in contrast to the well-documented findings of the light microscope.[1]

The purpose of the present study is to assess the effect of a unipolar (UP) electrocoagulation system and a bipolar (BP) coagulation system on human and albino rat peripheral nerves located adjacent to the area of coagulation. We also tried to elucidate the differences in detrimental effects of UP versus BP coagulation on neural tissue. We disregarded the coagulative effects of both BP and UP methods and concentrated on their possible harmful effects.

MATERIALS AND METHODS

Six human greater auricular nerves were used to examine the ultrastructural alterations induced by the UP system and to compare the effects to those of the BP system. The nerves were obtained from dissected anatomic blocks from radical neck dissection and superficial parotidectomy. Three patients had metastatic cervical disease and the other three had benign parotid gland tumors. The patients had neither received previous irradiation treatment nor undergone neck operations in the area of the greater auricular nerve.

An albino rat was used as the animal model. After full halothane anesthesia was administered, the femoral nerve was exposed by means of sterile operative technique. The animal was painlessly sacrificed at the end of the operative procedure.

The human and albino rat nerves were divided into three groups: controls, UP-treated, and BP-treated. Segments of both human and albino rat nerves were randomly selected and in situ UP or BP spot-coagulated with a standard Valleylab electrosurgical unit. The nerves were coagulated for 2 seconds at a constant power setting of 50 W by means of a 30-gauge needle electrode for UP coagulation, and 60 W by means of microbipolar forceps for BP coagulation, producing satisfactory bleeding control. All surgery and testing were performed by the same surgeons (Y.Z. and R.S.).

The electron microscopic examination was performed, at the site of coagulation, on small pieces of dissected nerves immediately fixed in cold 1% glutaraldehyde in phosphate buffer, pH 7.4, postfixed in osmium tetroxide, dehydrated in graded alcohols, and embedded in Epon 812. Thin sections cut with an LKB Ultratom III were stained with uranyl acetate and lead citrate and examined with a Philips 300 transmission electron microscope at an acceleration voltage of 60 kV. All the electron microscope work was performed by the same investigator (M.D.).

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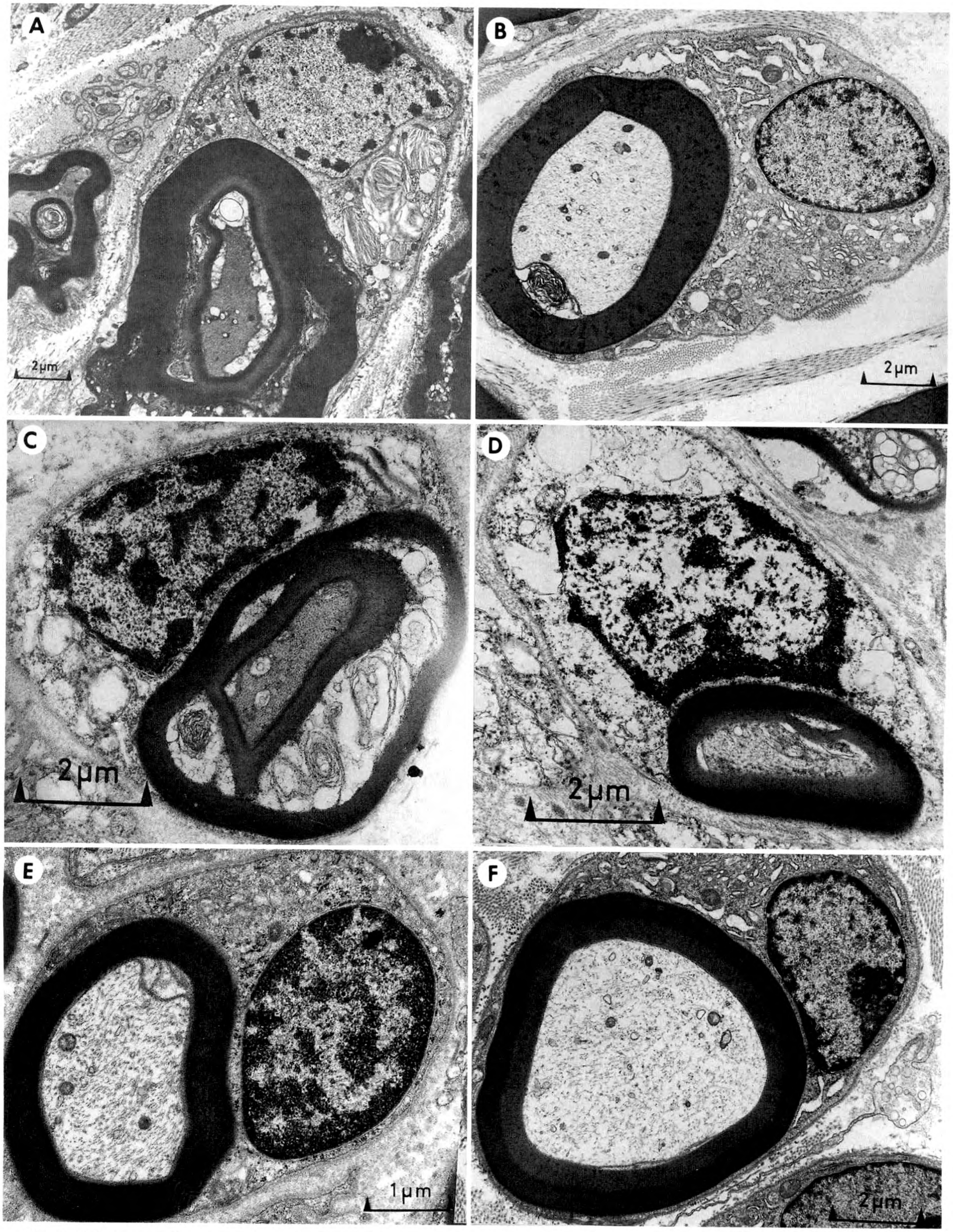

Fig 1. Schwann cells of myelinated nerves. In both human (A) and rat (B) control cells, nucleus is well preserved, cytoplasmic organelles appear normal, and myelin sheaths are intact. Following unipolar coagulation, Schwann cells in human (C) and rat (D) nerves show marked damage of nucleus, as well as of cytoplasmic organelles. On other hand, after bipolar coagulation, Schwann cells in human (E) and rat (F) nerves appear intact.

RESULTS

Myelinated Nerves. The control myelinated nerves in both humans (Fig 1A) and the albino rat (Fig 1B) contained Schwann cells characterized by their round

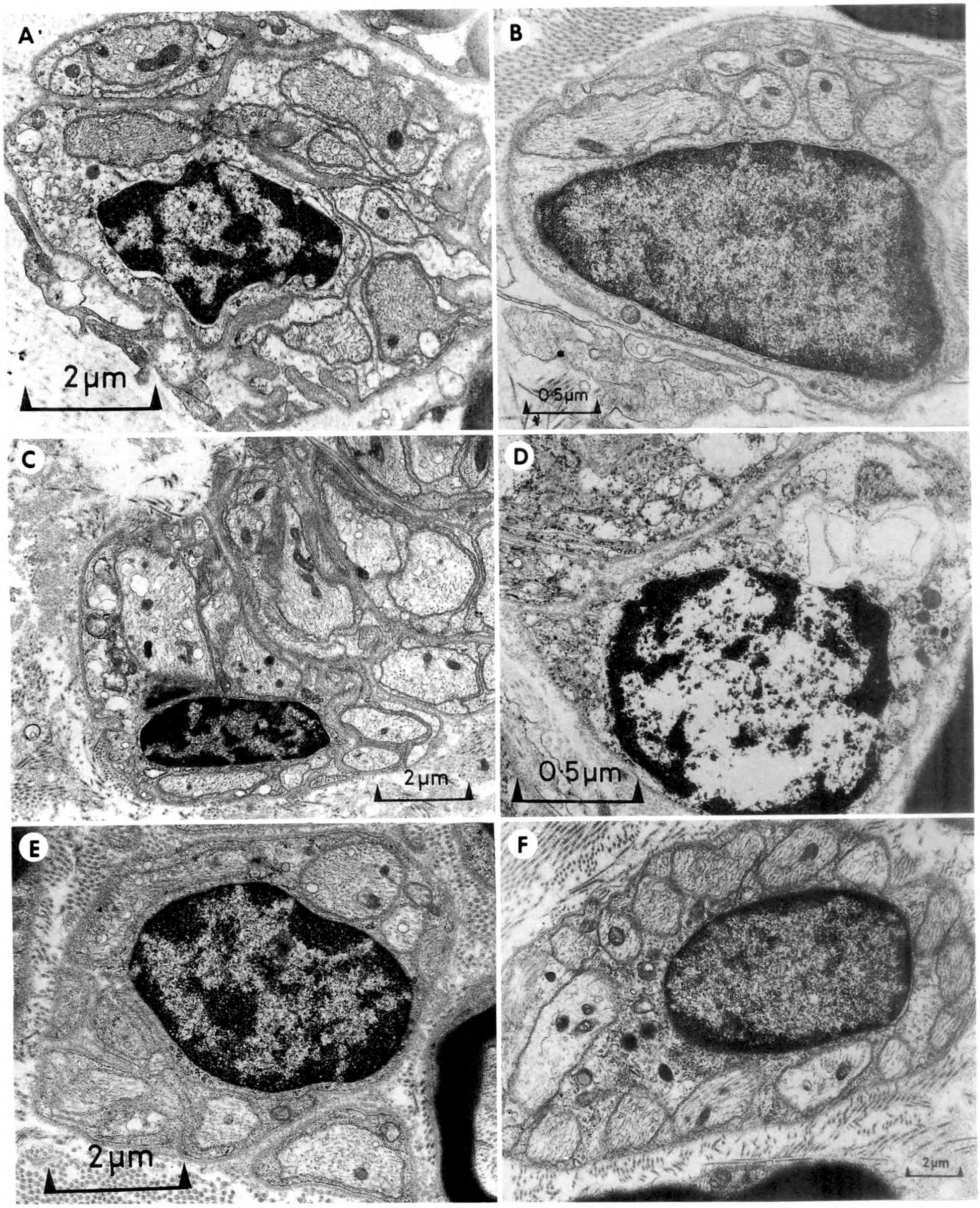

Fig 2. Schwann cells of unmyelinated nerves. Cells in human (A) and rat (B) control nerves show well-preserved nucleus and normal axons. Unipolar coagulation caused damage in both human (C) and rat (D) cells, more expressed in latter. Nucleus appears disrupted, and heterochromatin and axons are markedly damaged. Treatment of nerves by bipolar coagulation did not cause any visible damage in human (E) or rat (F) cells.

or oval nuclei, a small nucleolus, and peripherally located, scanty heterochromatin. The envelope of the myelin sheath was well preserved, and at higher magnification, numerous filaments, microtubules, and dense-cored vesicles were noted. The cell cytoplasm contained well-preserved round or oval mitochondria and cisternae of endoplasmic reticulum.

Following UP coagulation, marked damage of the Schwann cell structure was observed in human (Fig

1C) and albino rat (Fig 1D) nerves. The nucleus was partially and even completely destroyed, the nuclear membrane was interrupted, and the cell organelles, especially the mitochondria, were totally destroyed. The myelin sheaths and axons appeared well preserved. On the other hand, in BP coagulation–treated nerves the Schwann cell architecture remained well preserved in both human (Fig 1E) and rat (Fig 1F) specimens.

Unmyelinated Nerves. The Schwann cells of control human (Fig 2A) and albino rat nerves (Fig 2B) showed a round or oval nucleus with a greater amount of peripherally located heterochromatin. The nucleolus was inconspicuous. The cytoplasm contained several axons surrounded by mesaxons that enveloped the nerve fibers. Mitochondria were rarely observed.

Unipolar coagulation caused damage to the Schwann cells in human (Fig 2C) as well as albino rat nerves (Fig 2D). The nucleus was severely affected, the nuclear cell membranes were grossly disrupted, and the axons were damaged almost beyond recognition.

However, following BP coagulation, the Schwann cells in human (Fig 2E) and albino rat (Fig 2F) nerves were well preserved and their structure did not differ from that of the controls.

DISCUSSION

For over 50 years, high-frequency electrosurgery has been used to cut or coagulate biologic tissue. However, it must be ensured that only the targeted tissue suffers thermal damage during coagulation in order to obtain hemostasis.

There has been a growing interest among surgeons in improving the quality of treatment for patients who undergo diathermy coagulation in an area close to peripheral motor nerves or cranial nerves and their branches (unpublished data, K.T. Le et al). In order to prevent undesirable effects of the electric current such as electrolysis and stimulation of nerves and muscles, an alternating current with a frequency of at least 300 kHz must be used for high-frequency surgery. When high-frequency surgery is used, the surgeon expects efficient hemostasis as a result of vessel coagulation. The efficiency with which bleeding can be stopped depends on the intensity of thermal coagulation. Biologic tissue can only be coagulated by thermal means if the requisite temperature of approximately 70°C has been obtained.

Thermal heating as a result of electrical flow is considered to be the primary mechanism of tissue damage in electrical injury. An alternative nonthermal mechanism of cellular injury occurring in electrical trauma is membrane electroporation,[2] which is the electrically driven formation of aqueous pores in the cellular lipid membrane. If the pores' radius enlarges, the membrane will rupture. This mechanism occurs quickly, long before heating becomes significant.

The employment of high-frequency alternating currents for the destruction of tissue is referred to as surgical diathermy or electrosurgery. Currents of different types and intensities are used. Alternating current is more destructive than direct current. High tension (a current of more than 1,000 V) is more destructive than low tension. The larger the site, the greater the injury.[3]

In electrosurgery the destruction of tissue is accomplished by fulguration, desiccation, coagulation, and electrocutting.[4] Fulguration is useful for destruction of superficial lesions. For this purpose the damped current of a long-wave diathermy apparatus is employed. The active electrode is held in close proximity to the lesion but not inserted into it. The tissue destruction is superficial and is brought about by dehydration and shrinkage, the result of the evaporation of the water content of the treated tissue.

Electrodesiccation uses the high-voltage current derived from the monoterminal of a conventional diathermy generator and is similar to fulguration, but the active needle electrode is inserted into the area to be destroyed.

Electrocoagulation causes a more extensive and deeper destruction of tissue than the above-mentioned forms of surgical diathermy. A damped high-frequency current is employed. The current is of high amperage and low voltage.

The depth and extent of destruction that results from UP coagulation depend on the type of electrode used, the strength of the current, and the duration of application. The tissue becomes a grayish mass, composed of cells whose normal structure has been destroyed, and hyalinization necrosis results.

Monopolar (UP) electrosurgical devices have one small electrode in the instrument that the surgeon holds, and the other electrode is a large plate beneath the patient. Whenever monopolar devices are activated, electrical currents are passed completely through the patient's body.

Bipolar electrosurgical devices have two electrodes, between which the radio frequency high electrical voltages are applied at the point of tissue cutting, and current passes only through the tissues between the BP electrodes.

Given the results obtained in our present transmission electron microscopy study, BP coagulation should be the method of choice in electrocoagulation in proximity to peripheral or cranial nerves.

REFERENCES

1. Hambley R, Hebda PA, Abell E, Cohen BA, Jegasothy BV. Wound healing of skin incisions produced by ultrasonically vibrating knife, scalpel, electrosurgery, and carbon dioxide laser. J Dermatol Surg Oncol 1988;14:1213-7.

2. Neumann E, Rosenbeck K. Permeability changes induced by electric impulses in vesicular membranes. J Membr Biol 1972;10:279-90.

3. Small A. The accelerated surgical treatment of electrical burns of the mouth in children. In: Sisson GA, Tardy ME, eds. Plastic and reconstructive surgery of the face and neck. New York, NY: Grune and Stratton, 1977:290-6.

4. Piersol GM. Physical medicine in otolaryngology. In: Maloney WH, ed. Otolaryngology. New York, NY: Harper & Row, 1972:1-21.

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SLEEP FLUOROSCOPY FOR LOCALIZATION OF UPPER AIRWAY OBSTRUCTION IN CHILDREN

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The management of children with upper airway obstruction (UAO) in whom previous airway surgeries or concomitant craniofacial or neuromuscular abnormalities exist is complicated by potential obstruction at multiple sites. Sleep fluoroscopy (SF) provides a dynamic representation of relative degrees of obstruction at multiple levels of the pediatric airway. Fifty-five SF studies were performed on 50 infants and children to localize obstructive sites. Correlation was assessed with findings on direct laryngoscopy and bronchoscopy under spontaneous ventilation. In 24 (44%), endoscopic and SF findings correlated exactly. The SF studies identified a site of UAO in 11 patients with normal findings on endoscopic examination and multiple sites of UAO in 16 others. Two thirds of these occurred at the hypopharynx and tongue base. The SF studies failed to detect 5 airway abnormalities in 4 patients. The sensitivity of SF for endoscopically verified laryngotracheal lesions was lowest for glottic (67%) and subglottic (70%) locations and higher for tracheal (92%) and supraglottic (100%) sites. Sleep fluoroscopy altered the course of treatment in 26 (52%) children. It appears to be a valuable adjunct to endoscopy in the identification and management of pediatric UAO when hypopharyngeal collapse or multiple levels of obstruction are suspected.

KEY WORDS — craniofacial anomaly, fluoroscopy, obstructive sleep apnea, upper airway obstruction.

INTRODUCTION

The otolaryngologist who cares for children is frequently called upon to evaluate and treat upper airway obstruction (UAO). Managing the child with a complex medical history, in whom the structural or functional integrity of the upper airway may be compromised at more than one site, presents a special diagnostic and therapeutic challenge. It may be difficult to appreciate multiple areas of obstruction and the relative impact of each pathologic site on total airway patency.

Children at risk for multilevel UAO include those with congenital craniofacial anomalies, neuromuscular disorders, or metabolic storage diseases.[1-4] The mucopolysaccharidosis of Hurler's syndrome, for example, is characterized by skeletal anomalies, such as mandibular hypoplasia, as well as soft tissue infiltration and enlargement of the nasopharyngeal, oropharyngeal, hypopharyngeal, and laryngeal soft tissues. Clinical evidence of UAO may represent obstructive disease at any, or all, of these airway sites.[2] A history of surgical manipulation of the airway can similarly complicate the diagnostic process.[5,6] Subsequent UAO may represent progression or residua of the original disorder, alteration of airflow mechanics, or sequelae of the surgery itself (eg, anterior tracheal wall collapse following tracheostomy).

Physical and endoscopic examinations will identify clearly the cause of UAO in the majority of children. Direct laryngoscopy and bronchoscopy (DLB) offers superior diagnostic visualization of the anatomy and, when spontaneous respiration is maintained, the physiology of the larynx and tracheobronchial tree. Rigid endoscopy is, however, more limited in its ability to determine functional obstruction by soft tissues of the nasopharynx, hypopharynx, or tongue base.[7-9] The patency and function of the pharyngeal and laryngeal airway is readily assessed with flexible fiberoptic endoscopes,[10-12] but safe airway examination distal to the subglottis is usually not possible in the infant or young child with UAO.[13-15] Performed separately or in tandem, these endoscopic techniques are routinely used to delineate individual foci of disease as airway sites are examined in sequence. In extraordinary cases, the site of greatest obstruction may still be unclear.

An imaging technique that allows all levels of the upper airway to be visualized simultaneously during respiration might augment the diagnostic information gleaned from endoscopy. Fluoroscopy provides a dynamic representation of the patency of the entire

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upper airway, from nasopharynx to carina, during both phases of the respiratory cycle.[16,17] In complex cases, supine fluoroscopic evaluation during sleep may enhance the likelihood of localizing multiple obstructive sites and improve the identification of UAO related to inspiratory collapse of soft tissues within the pediatric airway.[17-19]

The purpose of this study is to evaluate the usefulness of sleep fluoroscopy (SF) in the localization and management of UAO in children. The study population comprises children in whom an underlying congenital or iatrogenic condition with a predisposition to UAO at more than one airway site exists. The value of SF as a diagnostic modality is assessed first by its ability to identify laryngeal and tracheal obstructive disease witnessed upon DLB, the current "gold standard" for laryngotracheal diagnostic investigation. The potential for SF to detect single or multiple sites of airway obstruction, irrespective of endoscopic findings, is then addressed through correlation of fluoroscopic and endoscopic findings at discrete sites within the upper airway (nasopharynx, hypopharynx, supraglottis, glottis, subglottis, and trachea) for each child. Management of UAO in these children was initially based on endoscopic findings alone. An estimation of SF's impact on patient management is made on the basis of the percentage of patients in whom the treatment plan was altered by the addition of SF findings.

MATERIALS AND METHODS

Infants and children under the age of 16 years were evaluated initially by a staff physician in the Department of Pediatric Otolaryngology at the Cincinnati Children's Hospital Medical Center. Patients with evidence of UAO on the basis of history and physical examination were evaluated further with airway soft tissue radiographs or flexible fiberoptic laryngoscopy (FFL) or both. Direct laryngoscopy and bronchoscopy under general anesthesia was performed when laryngeal or tracheal disease was suspected or when the severity of airway obstruction was not explained by FFL findings alone. Polysomnography was obtained for documentation of obstruction prior to endoscopy when symptoms occurred primarily during sleep.

Endoscopy was performed with the Hopkins system of rigid fiberoptic telescopes with a camera attachment for video recording. General anesthesia was induced via an inhalational technique without paralytic agents to allow dynamic airway characteristics such as respiratory phase variability and vocal cord mobility to be most accurately assessed. If a tracheostomy was present at the time of endoscopy, the examination included tracheoscopy with the tracheostomy tube in situ and with the tube withdrawn.

Patients were referred for evaluation of the airway by SF if one or more of the following conditions was present: 1) potential for obstruction at more than one site within the upper airway on the basis of an underlying congenital anomaly (craniofacial, neuromuscular, or metabolic), 2) potential for obstruction at more than one site within the upper airway due to previous airway surgery, 3) failure to improve clinically after a single site of obstruction was identified endoscopically and appropriately treated, or 4) persistent signs and symptoms of UAO despite normal findings on FFL and subsequent DLB.

Technique of Sleep Fluoroscopy. Sleep fluoroscopy was performed by a staff pediatric radiologist. An otolaryngologist was present for patients with a tracheostomy, to occlude or remove the tube during the study. Parents were allowed to attend the procedure in order to reduce patient anxiety and to verify that the observed breathing pattern was similar to that which had been observed at home. Food and drink were withheld for 4 to 8 hours prior to the study to minimize the risk of aspiration. Sleep was induced with oral chloral hydrate (70 to 100 mg/kg) or intravenous pentobarbital sodium (2 to 4 mg/kg). Each child was monitored continuously for respiratory rate, heart rate and rhythm, and blood oxygen saturation via transcutaneous pulse oximetry. Direct observations made by the radiologist included upper respiratory tract effort, thoracic wall motion, and episodes of apnea. Simultaneous audiovisual videotaping of the fluoroscopic examination allowed retrospective review and correlation of stridor or stertor with the visualized obstruction.

Fluoroscopy was performed in the lateral plane centering on three sites: oropharynx (at the level of the velum), hypopharynx, and intrathoracic trachea. In infants and children, this allows visualization of the airway from the nasopharynx to the distal trachea. When signs of obstruction occurred, fluoroscopy was recorded for 10 to 20 seconds at each of the three sites. The procedure was completed in approximately 1 hour (30 minutes for adequate sedation, 1 minute total fluoroscopy time, and 30 minutes recovery).

RESULTS

Fifty infants and children underwent 55 DLB and SF evaluations for UAO. Three children were studied twice and 1 child underwent 3 separate studies over a 20-month period. In these 4 children, repeat DLB and SF were warranted by persistent or recurrent UAO following operative intervention. Patient age at

TABLE 1. CONGENITAL SKELETAL, NEUROMUSCULAR, AND METABOLIC DISORDERS IDENTIFIED IN STUDY GROUP

	No. of Patients
Craniofacial	
Down syndrome	7
Choanal atresia	5
Treacher Collins syndrome	2
Pierre Robin syndrome	1
Crouzon's syndrome	1
Neuromuscular	
Hypotonia	6
Cerebral palsy	4
Arthrogryposis	1
Metabolic and other	
Hurler's syndrome	2
Nonsyndromic macroglossia	2
Total	31

TABLE 2. PRIOR PROCEDURES IN STUDY CHILDREN WITH HISTORY OF UPPER AIRWAY SURGERY

Procedure	*No. of Procedures*
Laryngotracheal reconstruction	16
Tracheostomy, closed	13
Tracheostomy, patent	11
Tonsillectomy and/or adenoidectomy	13
Arytenoidectomy	3
Epiglottoplasty	2
Repaired tracheoesophageal fistula	2
Repaired oropharyngeal stenosis	2
Excised laryngeal neurofibroma	1
Uvulopalatopharyngoplasty	1
Palatoplasty	1
Total	65

the time of evaluation ranged from 1 month to 15 years, with a mean of 3 years 9 months. None of the SF studies was associated with acute respiratory distress or other significant complication.

Congenital skeletal, neuromuscular, and/or metabolic disorders were present in 31 children. Of the 50 patients studied, 16 had an identifiable craniofacial anomaly such as Down syndrome or Pierre Robin anomalad. Eleven children demonstrated generalized neuromuscular abnormalities. Macroglossia was noted in 4 children: in 2 it was a manifestation of Hurler's syndrome, and in 2 others it was of nonsyndromic causation. Specific congenital disorders identified in the study population are listed in Table 1.

Twenty-two children had undergone no surgical alteration of the upper airway prior to SF and endoscopy. The remaining 28 shared a collective history of 65 airway-related surgeries excluding laryngoscopy and bronchoscopy. Eleven children were tracheostomy-dependent at the time of evaluation. Another 13 previously had undergone tracheostomy but were decannulated prior to SF and DLB. A complete list of procedures for patients with a history of upper airway surgery is provided in Table 2. Fifteen patients had both an underlying craniofacial malformation or neuromuscular disorder and a previous iatrogenic alteration of the airway.

Fifteen patients were referred for SF on the basis of clinical suspicion of UAO but with no significant airway disorder identified on FFL and DLB.

Sensitivity of Sleep Fluoroscopy for Laryngotracheal Lesions. On 55 laryngoscopic and bronchoscopic examinations, laryngeal or tracheal disease contributing to UAO was noted in 35. In 9 cases, supraglottic abnormalities (primarily those associated with laryngomalacia) were found. Sleep fluoroscopy localized airway obstruction to this site in all 9 patients. This represents a sensitivity of SF for endoscopically verified supraglottic disease of 100% for this site. At the level of the glottis, DLB identified an obstructing lesion in 3 patients with 1 case each of vocal cord paralysis, vocal cord granulomas, and glottic stenosis. The SF identified glottic obstruction in the latter 2 patients, but failed to detect immobility of the vocal folds. The sensitivity of SF for this limited number of glottic lesions therefore approaches 67%. Subglottic stenosis was found on 10 DLBs with obstruction localizing to this level by SF on 7 occasions (70%). Within the trachea, suprastomal collapse on inspiration in patients with previous tracheostomy was noted on DLB in 9 patients. Four other endoscopic examinations revealed tracheal obstruction including diffuse tracheomalacia and innominate artery compression. Sleep fluoroscopy identified these same lesions in 12 of the 13, a sensitivity of 92% for tracheal disease.

Correlation of Sleep Fluoroscopy and Endoscopic Findings. Table 3 outlines the general correlation between normal and abnormal endoscopic and SF studies. In 4 children, both DLB and SF findings were normal. Thirty-six DLB examinations with abnormal findings had a corresponding SF study that also had abnormal findings. Of these 36, 20 correlated exactly with the same site or sites identified by both methods. In 18 instances, the same single site was identified by both DLB and SF, and in 2 patients multiple sites of obstruction correlated exactly between the two methods of study. Therefore, a total of 24 (4 normal and 20 abnormal) endoscopies and fluoroscopies correlated exactly. In the remaining 16 with abnormal endoscopic findings, a single site of obstruction was noted on DLB and multiple sites were identified by fluoros-

TABLE 3. CORRELATION OF RESULTS OF SLEEP FLUOROSCOPY AND ENDOSCOPY IN 55 STUDIES

	Normal on SF	*Abnormal on SF*
Normal on DLB	4	11
Abnormal on DLB	4	36

DLB — direct laryngoscopy and bronchoscopy, SF — sleep fluoroscopy.

TABLE 4. CORRELATION OF SITES OF OBSTRUCTION IDENTIFIED BY SF AND ENDOSCOPY

Site	*Identified on Both DLB and SF*	*Identified on DLB and Not SF*	*Identified on SF and Not DLB*	*Total No. of Times Site Identified*
Nasopharynx	0	0	3	3
Hypopharynx	8	0	18	26
Supraglottis	9	0	2	11
Glottis	2	1	0	3
Subglottis	7	3	0	10
Trachea	12	1	6	19
Total	38	5	29	72

SF — sleep fluoroscopy, DLB — direct laryngoscopy and bronchoscopy.

copy.

Eleven cases in which no abnormality was identified on DLB demonstrated at least 1 obstructive site on SF. Obstruction in two thirds (8) of these children occurred in the region of the hypopharynx and tongue base. Inspiratory tracheal and nasopharyngeal obstruction were also observed on SF. Sleep fluoroscopy failed to localize 5 sites of UAO in 4 patients with laryngotracheal lesions demonstrated on DLB. Table 4 details the number of times a particular airway site was identified as a site of obstruction by DLB and by SF. Since multiple sites of obstruction were identified in 21 studies on 18 patients, the numeric values exceed the number of studies performed. Seventy-two separate sites of UAO were identified in 51 DLBs and 51 SF studies (4 of the 55 airway evaluations were determined to be normal by both DLB and SF, ie, no obstructive sites identified). Multiple sites of airway obstruction were found in 41% of this patient population.

Influence of Sleep Fluoroscopy Findings on Patient Management. Localization of UAO by SF altered the course of treatment, initially planned on the basis of clinical and endoscopic findings, in 26 children (52% of the study group). In 17, SF influenced the performance of a surgical procedure not anticipated by DLB, including intervention when no surgery was initially entertained or substitution of one surgical procedure for another to address a separate airway site. Sleep fluoroscopy obviated the need for surgery in 3 patients in whom DLB identified an abnormality but inaccurately estimated the degree of functional obstruction relative to other airway sites.

The findings on SF confirmed the treatment plan in the remaining 24 patients. Proceeding with surgical management was supported in 14 children. Deferment of operative intervention was supported by SF findings in 10 others.

DISCUSSION

Several types of radiographic imaging have been employed in the evaluation of UAO. Attempts to study the upper airway by static imaging modalities, such as cephalometric radiographs, computed tomography, magnetic resonance imaging, and acoustic reflection studies, have been limited to the visualization of fixed obstruction.[16,17,20] Fluoroscopy offers the advantage of a dynamic representation of the entire upper airway during both phases of respiration. Early work in the area of airway fluoroscopy focused on adults with obstructive sleep apnea (OSA) and originated in 1967 when Schwartz and Escande[21] studied the airflow patterns of a single pickwickian subject. Fluoroscopic evaluation was found to be well suited to sleep-related breathing disorders, since neither the positioning nor the equipment prevents the subject from attaining sleep and the functional effects of soft tissue collapse on the upper airway can be appreciated easily. Simultaneous polysomnography and fluoroscopy, termed somnofluoroscopy, correlates sleep stage with airway obstruction and has been used by several groups to study the pathophysiology of OSA in the adult population.[19,22,23]

Fluoroscopic evaluation of the pediatric airway was first described in a brief case report by Smith et al[18] in 1978. They utilized this modality to diagnose occlusion of the pharyngeal airway by the tongue base during sleep in a 12-year-old achondroplastic boy. One of the current authors in 1991 used SF to evaluate the airway in 2 children with Hurler's syndrome.[2] Although these sporadic case reports have alluded to its value, the literature presently lacks reports of large series addressing whether SF has a significant role in the management of pediatric UAO.

In this study, we found SF to be a useful supplement to DLB in the localization of pediatric UAO. It is not, however, an acceptable substitute for diagnostic airway endoscopy. The ability of SF to detect endoscopically documented laryngotracheal disorders was low for glottic and subglottic lesions (67% and 70%, respectively). These are typically fixed, adynamic obstructions such as vocal cord paralysis and subglottic stenosis. Since fluoroscopy best demonstrates changes in the caliber of the airway silhouette during the respiratory cycle, it has a limited ability to localize static obstructions in these locations. Plain anteroposterior and lateral neck and chest radiographs will often suffice to detect fixed narrowing of the subglottic larynx and trachea. The sensitivity of SF for dynamic obstruction, however, as occurs with laryngomalacia or tracheomalacia, was significantly higher (92% for tracheal lesions and 100% for supraglottic). These findings indicate that SF is an appropriate tool for detecting some types of obstructive disease.

More clinically significant is the apparent ability of SF to detect areas of obstruction not readily manifest on DLB. In 27 instances, SF identified a site of obstruction not diagnosed by DLB. As was the case specifically with laryngotracheal disease, SF appears best at identifying dynamic obstruction regardless of airway site and indeed appears superior to DLB in identifying inspiratory hypopharyngeal and tracheostomal collapse. The frequent finding of anterior tracheal obstruction reflects the high proportion of these patients in whom tracheostomy had been required.

Our initial premise that this study population is at risk for UAO at many levels of the airway was supported by the finding of multiple obstructive sites in 21 of 55 studies. In only 2 of these patients was obstruction localized to multiple sites by DLB alone. The ability to evaluate the entire upper airway simultaneously during fluoroscopy seemed to offer a distinct advantage. Not only was the multiplicity of obstructive sites easily appreciated, but when obstruction did occur at more than one site, a direct comparison of the relative degree of compromise at each could easily be made. A particularly useful application of SF therefore appears to be in the search for multiple levels of obstruction in a single patient.

The ability of SF to localize sites of UAO would have little relevance if that information is not helpful in directing treatment. In this group of patients, we found data obtained by this imaging modality to have a direct impact on patient care. Sleep fluoroscopy was performed in 15 cases in which clinical assessment suggested UAO but no cause was found on endoscopy. Four of these patients had normal findings on SF, affirming a plan of observation without intervention. A site of obstruction was localized by SF in the remaining 11 cases that guided the formulation of an appropriate treatment plan. In total, the management of 26 patients was changed by information supplied by SF. As an example, 1 child with Down syndrome, subglottic stenosis, and stomal collapse would have undergone laryngotracheoplasty with closure of the tracheostomy had not SF disclosed a greater obstruction at the level of the tongue base. Five children were spared an attempt at tracheostomy decannulation that was unlikely to succeed without reconstruction when SF demonstrated severe inspiratory collapse above the tracheostomy when the tube was removed. The successful management of UAO will be accomplished through careful history, physical examination, plain radiography, and endoscopy in the vast majority of children, but we found SF to be a helpful adjunct in the care of this particular patient population.

The modality of SF is not, however, without its drawbacks. The most frequently cited disadvantage to the use of fluoroscopy in children is the delivery of radiation.[8,16,17] Although the actual scanning time is brief (1 minute in total by our protocol), no dosing guidelines or limitations for diagnostic radiographic procedures are available for review. Somnofluoroscopy as described by Pepin et al[22] delivered 1 to 2 mGy of radiation per minute. No adverse acute or long-term effects have been reported to date for fluoroscopy administered in this fashion.

The second significant disadvantage is the need for sedation in order to provide a detailed study in small children and to reveal obstruction that may occur primarily during sleep. Fluoroscopy can be achieved in adults by enforcing sleep deprivation prior to the study,[22] but this approach may not be routinely applicable to pediatric subjects. Heavy sedation, particularly with narcotics, has the potential to produce central apnea by depressing respiratory drive. Central apnea can easily be distinguished from obstructive apnea by the absence of respiratory effort and was not observed in our patient group. The more critical issue is whether sedation causes a falsely high incidence of hypotonic hypopharyngeal and tongue base obstruction. While the central nervous system is exquisitely sensitive to barbiturates at sedative or hypnotic doses, skeletal and smooth muscle are not directly affected at the low dosages required to promote sleep in the absence of tactile stimulation.[24] All of our patients had signs or symptoms of UAO prior to sedation, and parents witnessing the SF study did not note significant worsening from their child's baseline respiratory status. When managing these children, particularly those with UAO in the face of normal findings on endoscopic examination, we felt it appropriate to accept fluoroscopic evidence of hypopharyngeal obstruction as clinically significant. It should not be surprising that UAO was often localized to the region of the hypopharynx and tongue base given this patient population, half (25/50) of whom had a syndrome associated with micrognathia, retrognathia, glossoptosis, macroglossia, or other congenital affliction of this anatomic area.

CONCLUSIONS

We found SF to be a relatively safe and useful procedure for examination of the pediatric upper airway. While the sensitivity of SF in identifying dynamic obstructive disease in the supraglottic larynx and trachea is acceptable, it identifies less well those lesions that tend to be fixed rather than functional, such as vocal cord paralysis and subglottic stenosis. Sleep fluoroscopy may therefore complement, but cannot replace, diagnostic airway endoscopy.

The advantages of SF appear to be the ability to 1) identify inspiratory collapse of the hypopharynx and tongue base and of the anterior tracheal wall and 2) assess multiple levels of UAO within a single patient. Our findings affirm that children with prior surgery or congenital anomalies affecting the upper airway, or both, are indeed at risk for UAO by these mechanisms. Information gleaned from SF influenced the management of half of our patient population; hence, the addition of SF findings to those obtained by endoscopy in these complex cases seemed to have significant clinical relevance. This modality proved most helpful when the severity of airway obstruction was unexplained by endoscopic findings or treatment of a single identifiable site failed to result in clinical improvement.

Sleep fluoroscopy appears to be a valuable adjunct to laryngoscopy and bronchoscopy in the localization and management of UAO in children in whom inspiratory hypopharyngeal collapse or involvement at multiple sites is likely.

REFERENCES

1. Marcus CL, Keens TG, Bautista DB, von Pechmann WS, Davidson Ward SL. Obstructive sleep apnea in children with Down syndrome. Pediatrics 1991;88:132-9.

2. Myer CM III. Airway obstruction in Hurler's syndrome: radiographic features. Int J Pediatr Otorhinolaryngol 1991;22:91-6.

3. Arnold JE, Allphin AL. Sleep apnea in the neurologically-impaired child. Ear Nose Throat J 1993;72:80-1.

4. Guilleminault C, Korobkin R, Winkle R. A review of 50 children with obstructive sleep apnea. Lung 1981;159:275-87.

5. Hoeve LJ, Rombout J. Pediatric laryngobronchoscopy. 1332 procedures stored in a data base. Int J Pediatr Otorhinolaryngol 1992;24:73-82.

6. Tom LWC, Miller L, Wetmore RF, Handler SD, Potsic WP. Endoscopic assessment in children with tracheotomies. Arch Otolaryngol Head Neck Surg 1993;119:321-4.

7. Guilleminault C, Stoohs R. Obstructive sleep apnea syndrome in children. Pediatrician 1990;17:46-51.

8. Brooks LJ. Diagnosis and pathophysiology of obstructive sleep apnea in children. Ear Nose Throat J 1993;72:58-60.

9. Guilleminault C. Obstructive sleep apnea syndrome and its treatment in children: areas of agreement and controversy. Pediatr Pulmonol 1987;3:429-36.

10. Rojewski TE, Schuller DE, Clark RW, Schmidt HS, Potts RE. Videoendoscopic determination of the mechanism of obstruction in obstructive sleep apnea. Otolaryngol Head Neck Surg 1984;92:127-31.

11. Wang D, Clement P, Kaufman L, Derde M. Fiberoptic examination of the nasal cavity and nasopharynx in children. Int J Pediatr Otorhinolaryngol 1992;24:35-44.

12. Yanagisawa E, Yanagisawa K. Endoscopic view of adenoids. Ear Nose Throat J 1994;73:74-5.

13. Holinger LD. Diagnostic endoscopy of the pediatric airway. Laryngoscope 1989;99:346-8.

14. Hawkins DB, Clark RW. Flexible laryngoscopy in neonates, infants, and young children. Ann Otol Rhinol Laryngol 1987;96:81-5.

15. aWengen DF, Probst RR, Frei FJ. Flexible laryngoscopy in neonates and infants: insertion through a median opening in the face mask. Int J Pediatr Otorhinolaryngol 1991;21:183-7.

16. Fleetham JA. Upper airway imaging in relation to obstructive sleep apnea. Clin Chest Med 1992;13:399-416.

17. Shepard JW, Gefter WB, Guilleminault C, et al. Evaluation of the upper airway in patients with obstructive sleep apnea. Sleep 1991;14:361-71.

18. Smith TH, Baska RE, Francisco CB, McCray GM, Kunz S. Sleep apnea syndrome: diagnosis of upper airway obstruction by fluoroscopy. J Pediatr 1978;93:891-2.

19. Walsh JK, Katsantonis GP, Schweitzer PK, Verde JN, Muehlbach M. Somnofluoroscopy: cineradiographic observation of obstructive sleep apnea. Sleep 1985;8:294-7.

20. Hoffman EA, Gefter WB. Multimodality imaging of the upper airway: MRI, MR spectroscopy, and ultrafast x-ray CT. Prog Clin Biol Res 1990;345:291-301.

21. Schwartz BA, Escande JP. Etude cineradiographique de la respiration hypnique pickwickienne. Rev Neurol 1967;87:677-8.

22. Pepin JL, Ferretti G, Veale D, et al. Somnofluoroscopy, computed tomography, and cephalometry in the assessment of the airway in obstructive sleep apnoea. Thorax 1992;47:150-6.

23. Katsantonis GP, Walsh JK. Somnofluoroscopy: its role in the selection of candidates for uvulopalatopharyngoplasty. Otolaryngol Head Neck Surg 1986;94:56-60.

24. Rall TW. Hypnotics and sedatives. In: Gilman AG, Rall TW, Nies AS, Taylor P, eds. The pharmacologic basis of therapeutics. 8th ed. Elmsford, NY: Pergamon Press, 1990:345-70.

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MENINGITIS AFTER ADENOIDECTOMY: AN ANATOMIC EXPLANATION

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Meningitis is a rare complication of adenoidectomy. During a 5-month period, two children at St Christopher's Hospital for Children developed meningitis within days following this surgical procedure. The potential causes of this complication that we investigated include coincidence, systemic hematogenous spread of bacteria to the central nervous system, and direct or indirect contamination of the cerebrospinal fluid by bacteria introduced by retropharyngeal injection of lidocaine hydrochloride and epinephrine. Based on statistical analysis of the available literature and anatomic studies of the pediatric nasopharyngeal region, we conclude that a retrograde flow of bacteria via a newly described anastomotic network of veins was the most likely cause of this sequela.

KEY WORDS — adenoidectomy, complication, head and neck anatomy, meningitis, vascular anatomy.

INTRODUCTION

Meningitis rarely complicates adenoidectomy. This is surprising, given the proximity of the nasopharynx (a highly contaminated field) to the central nervous system (CNS) and the exposure of a rich vascular bed during adenoid removal. In the years 1992-1993 more than 800 children underwent adenoidectomy with or without tonsillectomy at St Christopher's Hospital for Children. During this period one surgeon (G.I.) used a technique of retropharyngeal injection of lidocaine hydrochloride–epinephrine as part of the adenoidectomy procedure to reduce intraoperative bleeding. This technique was abandoned after two children, in a 5-month period, developed bacterial meningitis in the days after surgery. Since discontinuation of the injection technique, there has been no more meningitis in this patient group.

Four potential explanations for these unusual complications were explored: 1) coincidence, 2) hematogenous spread due to the adenoidectomy itself, 3) direct perforation into the subarachnoid space by the hypodermic needle, and 4) retrograde venous contamination of the cerebrospinal fluid (CSF) during rapid injection of the lidocaine and epinephrine. To determine the likelihood of each of these possibilities, a review of the literature on complications of adenoidectomy was undertaken, other surgeons with experience in nasopharyngeal injection were contacted by telephone, and two sets of anatomic studies were pursued. The results of this work and our proposed mechanism for meningitis after adenoidectomy are presented.

METHODS AND MATERIAL

Injection Technique. In each case, the patient, under general endotracheal anesthesia, was placed in Rose's position and the mouth was opened with a McIvor gag in preparation for adenoidectomy. The soft palate was retracted to reveal the lower edge of the adenoid bed. A 3-mL syringe and 27-gauge needle were used to inject 1 mL of sterile 1% lidocaine hydrochloride and 1:100,000 epinephrine per year of age with a maximum dose of 5 mL. The needle was passed through the adenoid bed under vision until it contacted the bone of the basicranium. It was then withdrawn a few millimeters to the presumed location of the retropharyngeal space. The plunger was retracted, and if no blood returned, injection was begun, ballooning out the retropharyngeal space. After 5 minutes the adenoidectomy was performed under indirect mirror guidance with a Barnhill curette and St Clair Thompson forceps. Hemostasis was obtained by compression with tonsillar tampons and by suction electrocautery.

Case Reports. Patient 1 was a 5-year-old boy who underwent an uneventful adenoidectomy and bilateral myringotomy with tube insertion for recurrent acute otitis media and nasal obstruction. Retropharyngeal injection of 5 mL of lidocaine hydrochloride with epinephrine was used. The patient was discharged from the hospital 4 hours postoperatively, alert and tolerating liquids. The following morning, he was noted to be lethargic and febrile to 38.2°C, and he vomited several times. He was admitted to the hospital, where a lumbar puncture was grossly puru-

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lent with 14,000 white blood cells (WBCs) and a glucose of 37 mg/dL. Culture of the CSF grew *Streptococcus pneumoniae*. He was treated with ceftriaxone sodium and dexamethasone and recovered without hearing loss or neurologic sequelae. Magnetic resonance imaging showed no evidence of a skull base defect.

Patient 2 was a 5-year-old girl who underwent an adenoidectomy for nasal obstruction in which retropharyngeal injection of 5 mL of lidocaine hydrochloride with epinephrine was used. She was discharged from the hospital 4 hours postoperatively, alert and tolerating liquids. The following day she developed fever, vomiting, and lethargy. Thirty-six hours postoperatively she was taken to the emergency room, where a lumbar puncture yielded CSF with 4,700 WBCs and a glucose of 16 mg/dL. Cultures grew *Haemophilus influenzae* type A. She was hospitalized and treated with ceftriaxone and dexamethasone and recovered without hearing loss or neurologic sequelae. Computed tomography showed no evidence of a skull base defect.

Anatomic Studies. To determine whether needle puncture of the subarachnoid space was possible via transoral injection, we recapitulated our injection technique in a morphologically normal 3-year-old child who had died of heart disease. With the fresh cadaver in Rose's position and a Dingman mouth gag in place, several attempts were made to reach the subarachnoid space by a peroral route. In addition to correct placement in the lower nasopharynx, the needle was passed "too low" in the oropharynx and "too high" in the nasopharynx, with lateral radiographic confirmation of their positions.

Fresh fetal cadaver experiments to determine the venous anatomy of the nasopharynx and cervical spine were performed as part of an orthopedic study and previously published in 1984.[1] These results were reanalyzed by the anatomist on that project (W.W.P.) to determine whether a pathway of collateral circulation between the nasopharyngeal submucosa and the CNS existed. In these experiments, two techniques were used. In the first set of experiments, vascular casts of the arterial and venous systems were created by injection of the vertebral arteries of third-trimester fresh fetuses. A solution of vinyl acetate was injected by syringe through a cannula in the ascending aorta. After the cannula was secured, the arch of the aorta was clamped just proximal to its junction with the ductus arteriosus in order to confine the flow of the medium to the branches of the arch. When the gingivae showed sufficient discoloration to indicate adequate penetration of the medium into the finer vessels, the cannula was clamped and the medium permitted to solidify for 24 hours. Due to numerous upper cervical arteriovenous anastomoses, the suboccipital epidural veins also became well injected. The upper cervical spine, the base of the skull, and the peripharyngeal tissues were then removed en bloc and were corroded in 10% solution of potassium hydroxide until the soft and hard tissues were completely digested. The remaining cast was then pruned of extraneous vessels to show the desired area. In the second set of experiments, pharyngeal venous drainage was studied in six third-trimester and full-term fetuses by means of retrograde injection of the azygos vein with India ink, synthetic latex, and water in a 2:1:1 ratio for a 2-hour period. The specimens were then embalmed with formalin (2%) and phenol (0.5%), bleached (1.5% hydrogen peroxide), decalcified (4% acetic acid), dehydrated (100% ethanol), and cleared (methyl salicylate). Specimens sectioned in the parasagittal plane were transilluminated, photographed, and evaluated.

Statistics. The probability of bacterial meningitis occurring by chance alone in two children in a 5-month period was calculated from an established study of the incidence of bacterial meningitis beyond the neonatal period and by assuming a Poisson distribution to model a rare event.

RESULTS

Literature Review. A computerized review of the literature revealed only seven reports of meningitis after tonsillectomy and/or adenoidectomy.[2-4] In at least one of these seven cases, the patient had a nasopharyngeal encephalocele that was not appreciated prior to adenoidectomy. Several series reporting the complications of more than a thousand adenotonsillectomies exist, and none of these includes a case of meningitis.[5-9] While an accurate denominator cannot be determined, it can be assumed safely that a case of meningitis is unlikely to occur in more than one in five thousand cases, and that the true incidence is probably much lower than this.

Population-based studies in the states of South Carolina, Minnesota, Vermont, and New Mexico quote the incidence of bacterial meningitis in children beyond the neonatal period at between 5 and 7 per 100,000 per year.[10] Assuming a Poisson distribution to model a rare event, with an expected incidence of 0.017 cases of meningitis in a 5-month period ($5/100{,}000 \times 800$ children $\times$ 5/12 year), we find the probability of two children contracting meningitis by chance alone is in the range of one in a million ($p < .000001$). The chance that each case should occur within 3 days of surgery by chance is much lower than this.

Anatomic Studies. Multiple attempts to pass a

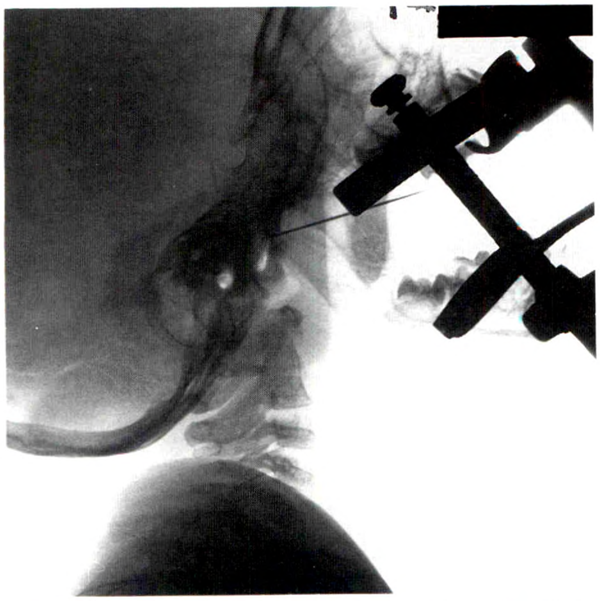

Fig 1. Lateral radiograph of fresh pediatric cadaver in Rose's position. Attempts at entry into subarachnoid space were unsuccessful at any angle. Needles passed through nasopharynx, as well as those misdirected through oropharynx, were stopped by solid basioccipital bone.

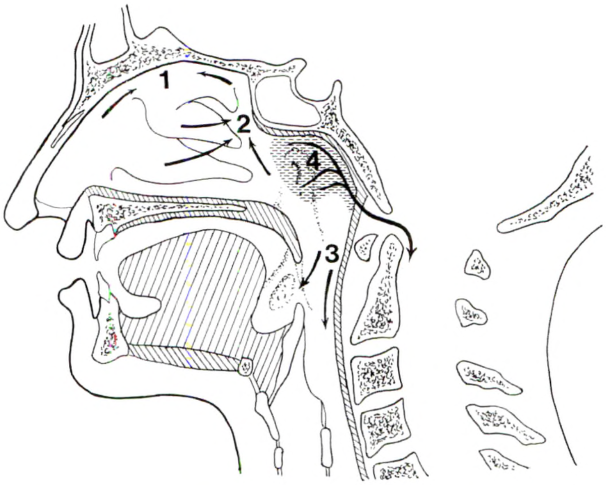

Fig 2. Schematic representation of venous drainage of nasal cavity, nasopharynx, and oropharynx. Drainage from upper part of lateral wall of nasal cavity goes into orbital veins through anterior and posterior ethmoidal veins (1), whereas drainage from lower part of lateral nasal wall and nasal septum goes to pterygoid plexus, deep in face, through tributaries of sphenopalatine veins (2). Oropharynx drains into tonsillar tributaries of maxillary veins and posterior pharyngeal plexus, as well as tonsillar branch of lingual vein (3). Through fine radicals of pharyngovertebral veins, posterior nasopharyngeal region (indicated by cross-hatching; 4), drains through pharyngobasilar fascia and anterior atlanto-occipital region to communicate with periodontoidal plexus and epidural veins. (Reprinted with permission.[1])

hypodermic needle into the subarachnoid space by a transoral approach were unsuccessful. The needle was repeatedly introduced at 0.5-cm intervals along an arc ranging from the high oropharynx to the hard palate. Even when passed at unusual angles and well above and below the prescribed site for nasopharyngeal injection, the needle's course was consistently blocked by the solid bone of the basicranium (Fig 1).

If we combine this information with data derived from the injection studies that provided both corrosion casts and parasagittal sections of the vascular anatomy of the nasopharynx and surrounding structures (Figs 2-4[1]), it is possible to reconstruct a route for retrograde venous flow from the nasopharynx submucosa to the subarachnoid space. The pharyngovertebral veins provide the most direct route for nasopharyngeal blood- or lymph-borne inclusions to gain access to structures related to the superior cervical vertebrae. Formed by radicals in the nasopharyngeal submucosa, these veins pass bilaterally on both sides of the medial pharyngeal bursa, in close approximation to the basilar process of the occipital bone, and communicate with the periodontoidal venous plexus, which is part of the epidural suboccipital venous sinus (of Zolnai) that is an expansion of the spinal epidural veins in the C1-2 region of the spinal canal. Small venous radicals connect this sinus complex with the surrounding osseous and connective tissues of the upper cervical region and also provide a transdural access to the leptomeninges. Although this latter passage may seem less obvious, it is readily demonstrable by fine dissection. The vertebral arteries are entirely surrounded by components of the suboccipital sinus where they penetrate the dura. These veins, at this point, receive fine venules from an intradural venous cuff that envelops the first several millimeters of the intradural segment of the vertebral arteries. Similarly, but on a finer scale, the upper cervical vertebral nerves show an epidural venous cuff that receives communications from the meningeal veins related to the venous drainage of the regional nerve roots and spinal cord. When it is considered that none of this venous system complex, from the nasopharyngeal radicals to the radiculomedullary veins, contains valves, the blood flow may be reciprocal at any point in response to pressure variations. Rapid injection of a solution contaminated by oral flora, or even a transient increase in vertebral epidural sinus pressure in response to a Valsalva maneuver, may momentarily drive epidural venous blood and its contaminants into the intradural meningeal regions. It is likely that such retrograde flow of blood occurs in the physiologic state, but is not a risk factor for meningitis, as this blood is sterile.

DISCUSSION

Meningitis is a rare complication of tonsillectomy or adenoidectomy. A computerized review of the

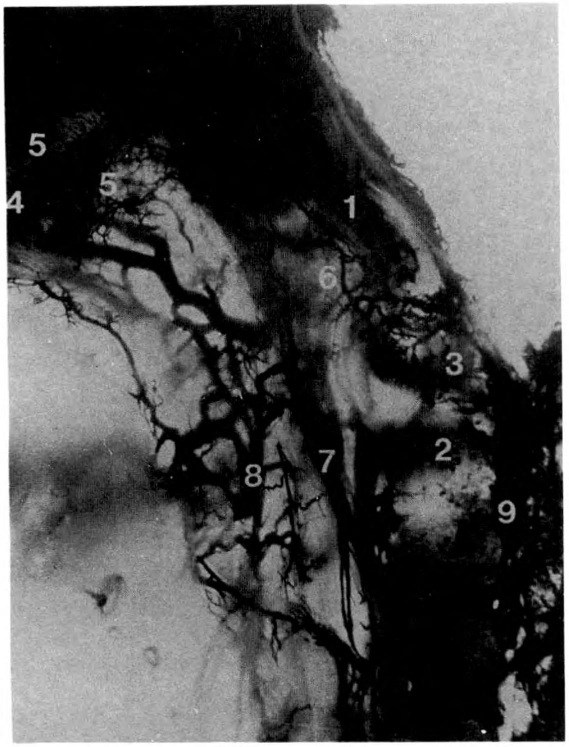

Fig 3. Photograph of cleared and transilluminated parasagittal section of lateral pharyngeal wall, basiocciput, and upper cervical vertebrae from fetus of 32 weeks' gestation. In this specimen, medium had filled jugular system of one side, as well as epidural venous sinuses. Branches of maxillary and pharyngeal veins (8) can be seen receiving tributaries from lateral walls of oropharynx and nasal cavities. Medium that filled epidural sinuses (9) passed in retrograde flow through periodontoidal plexus that envelops odontoid process (3). Medium also passed anterosuperiorly through pharyngovertebral vein (6) lying ventral to basiocciput (1) to fill fine plexus (5) surrounding torus tubarius (4). Posterior pharyngeal wall (7) delineates retropharyngeal cleft between it and body of axis (2). (Reprinted with permission.[1])

world's literature revealed only seven cases. This is surprising, given the level of bacterial contamination in the nasopharynx and the known 40% incidence of bacteremia following tonsillectomy and adenoidectomy.[11,12] When our first child developed meningitis 3 days after surgery, we were concerned, but felt that it was either a chance event or the result of spread of organisms from the bloodstream to the CNS. A chance explanation could not be invoked when a second case occurred in short order. This is supported by our literature search and analysis suggesting that two episodes of meningitis in 5 months in a population of 800 otherwise healthy children is less than a one in a million occurrence.

Aberrant communications between the nasopharynx and subarachnoid space, especially nasopharyngeal encephaloceles, have been associated with meningitis after adenoidectomy.[4,13] Both of our patients were studied and had normal skull bases.

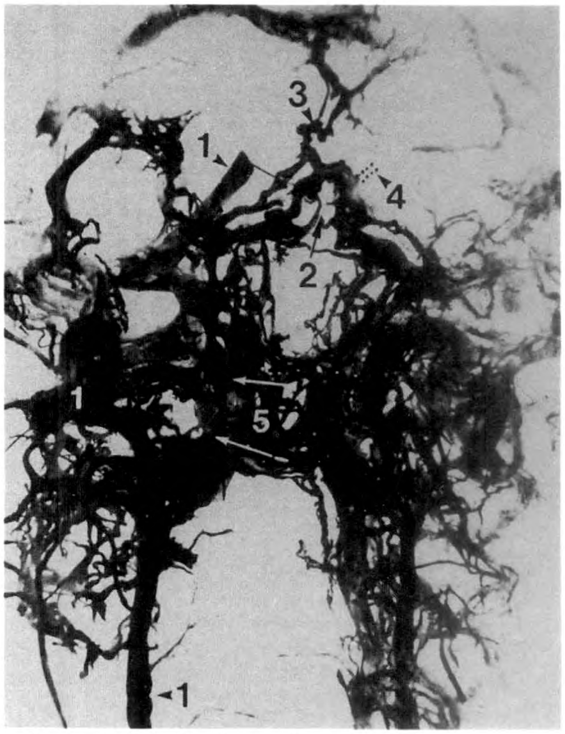

Fig 4. Photograph of posterior aspect of plastic cast of upper cervical veins and arteries from term fetus. Medium had entered region by vertebral arteries (1), but had also filled upper cervical epidural veins by way of numerous arteriovenous anastomoses characteristic of this area. Left pharyngovertebral veins (3) are shown connecting to periodontoidal plexus that outlines "ghost" of corroded odontoid process (2). Similar vein on other side of midline (4), indicated by dots, was broken in corrosion process (2). Posterior and ascending artery of axis that supplies odontoid is indicated by two white arrows (5). (Reprinted with permission.[1])

We must assume that CNS contamination in our patients resulted from the injection technique used. Our cadaver study shows that direct penetration of the subarachnoid space by a needle inserted into the adenoid bed is not possible. This leaves retrograde venous flow of contaminated injection fluid as the most likely mechanism. Fetal injection studies clearly demonstrate that valveless venous anastomoses exist extending from the nasopharyngeal submucosa to the subarachnoid space. The pharyngovertebral veins penetrate the anterior atlanto-occipital membrane to communicate with the suboccipital venous sinus (of Zolnai) that is an expansion of the spinal epidural veins in the C1-2 region of the spinal canal.[14,15] Small venous radicals connect this sinus complex with the surrounding osseous and connective tissues of the upper cervical region and provide access to the leptomeninges.

Retropharyngeal injection of vasoconstrictive agents during adenoidectomy or prior to the resection of vascular tumors is not new. Centers in the United States, Australia, and Germany[16] (also B. Benjamin and D. Parsons, personal communication, 1995) continue to use this technique, at least in selected cases. While injection of vasoconstrictor clearly decreased intraoperative bleeding and improved visualization during adenoidectomy, we abandoned this approach in 1993 and have had no additional cases of meningitis since. Injection of the vascular soft tissues of the head has been associated with ophthalmic and intracranial complications in the past.[17,18] Given this newly demonstrated vascular pathway to the CNS, retropharyngeal injection of anesthetic agents should be avoided unless absolutely necessary for control of bleeding. Care to avoid intravascular injection and slow injection of the minimum volume of agent may reduce the risk of retrograde venous flow and CNS contamination.

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REFERENCES

1. Parke WW, Rothman RH, Brown MD. The pharyngovertebral veins: an anatomical rationale for Grisel's syndrome. J Bone Joint Surg 1984;66:568-74.

2. Batrakov N. Meningitis following tonsillectomy. Vestn Otorinolaringol 1964;26:92-5.

3. Singer JI. Evaluation of the patient with neck complaints following tonsillectomy or adenoidectomy. Pediatr Emerg Care 1992;8:276-9.

4. Richter E, Pirsig W, Tanzer A. Offener Canalis craniopharyngeus mit spheno-pharyngealer Meningoenzephalozele. HNO 1975;23:240-5.

5. Tolczynski B. Tonsillectomy; its hazards and their prevention. Eye Ear Nose Throat Monthly 1969;48:71-80.

6. Tate N. Deaths from tonsillectomy. Lancet 1963;7:1090-1.

7. Rasmussen N. Complications of tonsillectomy and adenoidectomy. Otolaryngol Clinic North Am 1987;20:383-90.

8. Pratt LW. Tonsillectomy and adenoidectomy: mortality and morbidity. Trans Am Acad Ophthalmol Otolaryngol 1970;74:1146-54.

9. Donaldson JD, Stool SE. Complications of tonsillectomy and adenoidectomy. In: Conley JJ, ed. Complications of head and neck surgery. Philadelphia, Pa: WB Saunders, 1979:227-38.

10. Feigin RD. Bacterial meningitis beyond the neonatal period. In: Feigin RD, Cherry JD, eds. Pediatric infectious diseases. 3rd ed. Philadelphia, Pa: WB Saunders, 1992:401-28.

11. François M, Bingen EH, Lambert-Zechovsky NY, Mariani-Kurkdjian P, Nottet J-B, Narcy P. Bacteremia during tonsillectomy. Arch Otolaryngol Head Neck Surg 1992;118:1229-31.

12. Van Eyck M. Bacteremia after tonsillectomy and adenoidectomy. Acta Otolaryngol (Stockh) 1976;81:242-3.

13. Kenna MA. Transsphenoidal encephalocele. Ann Otol Rhinol Laryngol 1985;94:520-2.

14. Parke WW, Valsamis MP. The ampulloglomerular organ: an unusual neurovascular complex in the suboccipital region. Anat Rec 1967;159:193-8.

15. Parke WW. The vascular relations of the upper cervical vertebrae. Orthop Clin North Am 1978;9:879-89.

16. Nessel E, Mundnich K. Surgery of the nasopharynx. In: Naumann HH, ed. Face and facial skull. Philadelphia, Pa: WB Saunders, 1980:107-31. (Head and neck surgery, indications, techniques, pitfalls; vol 2.)

17. Mabry RL. Visual loss after intranasal corticosteroid injection. Arch Otolaryngol 1981;107:484-6.

18. McCleve DE, Goldstein JC. Blindness secondary to injections in the nose, mouth and face: causes and prevention. Ear Nose Throat J 1995;74:182-8.

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Ann Otol Rhinol Laryngol 105:1996

ELECTRICAL PACING OF THE PARALYZED HUMAN LARYNX

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This study represents the first attempt to electrically pace the paralyzed human larynx. The goal was to determine if electrical stimulation of the posterior cricoarytenoid muscle could produce functional abduction of the vocal fold in pace with inspiration. An external apparatus was used to sense inspiration and reanimate the unilaterally paralyzed larynx of a thyroplasty patient. Stimuli were delivered through a needle electrode to locate and pace the abductor muscle. The magnitude of electrically induced abduction was comparable to spontaneous movement on the normal side. The abduction was appropriately timed with inspiration; this finding demonstrated that this simple pacing system could effectively modulate stimulation with patient respiration.

KEY WORDS — functional neuromuscular stimulation, human, laryngeal pacing, muscle paralysis, posterior cricoarytenoid muscle.

INTRODUCTION

Recurrent laryngeal nerve injury compromises both abductory and adductory function of the vocal folds. In case of bilateral nerve damage, both folds are paralyzed in the paramedian position, severely restricting ventilation. The loss of abduction often requires emergency tracheotomy to create a functional airway.

Conventional methods of treatment for bilateral laryngeal paralysis are less than ideal. Surgical therapies such as arytenoidectomy, arytenoidopexy, or vocal fold resection enlarge the glottis, but jeopardize voice and airway protection. These procedures are also destructive and irreversible, and tend to ignore the long-term effects of atrophy on vocal fold mass and position. Consequently, efforts have been directed toward a more physiologic approach to rehabilitation that can reanimate the muscles of the larynx, prevent denervation atrophy, and restore vocal fold abduction. One such innovative approach is laryngeal pacing. This strategy involves electrical stimulation of the posterior cricoarytenoid (PCA) muscles during the inspiratory phase of respiration to abduct the vocal folds. During noninspiratory phases, the folds passively relax to the midline for voicing and deglutition.

Use of functional neuromuscular stimulation (FNS) to reanimate laryngeal muscles was conceived and introduced into the field of otolaryngology by Zealear and Dedo.[1] Subsequent studies of abductor pacing have been conducted in acute animal preparations. Obert et al[2] stimulated PCA muscles to restore full abduction in bilaterally denervated canines, and suggested that stimulation be synchronized with inspiration. Bergmann et al[3] demonstrated the plausibility of respiratory regulation of stimuli, using a chest wall sensor to trigger stimulation of canine abductors. Other investigators have explored different sources of inspiratory drive[4-6] (also Herzon and Zealear, unpublished observations) and determined that changes in intrathoracic pressure appeared to offer the most reliable trigger signal. Sanders[7] systematically evaluated the stimulus-response characteristics of chemically denervated canine PCA muscles. Recently, Zealear et al[8] described a stimulus delivery system and optimum stimulus parameters for activation of chronically denervated PCA muscles.

A paucity of studies have addressed the challenge of chronic electrical pacing. Zrunek et al[9] described continuous pulsed stimulation of the denervated PCA muscle in sheep. Muscle contractility and evoked abduction were maintained with stimulation over a 2-month period. Zealear et al (unpublished observations) explored the effects of continuous train stimulation on the chronically denervated canine PCA muscle. Significant changes in muscle physiology, biochemistry, and the pattern of reinnervation were observed.

To date, there has been no study of electrical pacing in the human. The objectives of the present study were 1) to determine if electrical stimulation of a paralyzed human PCA muscle would result in

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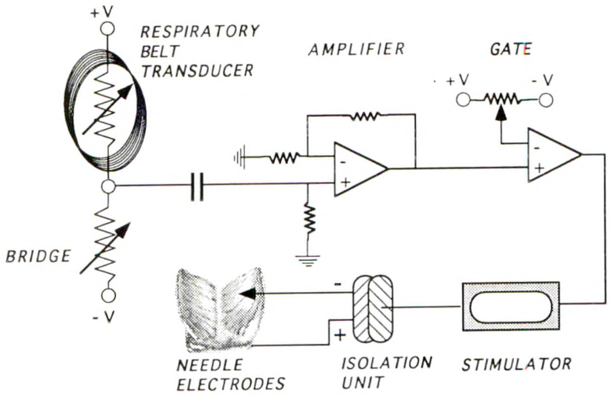

Fig 1. Schematic of external pacing system. Respiratory transducer had sufficient sensitivity to sense chest wall expansion against closed glottis. For prolonged or chronic pacing sessions, stimulator was equipped to deliver charge-balanced biphasic pulses. Under such conditions, bipolar stimulation would be administered with both electrodes in posterior cricoarytenoid muscle to prevent activation of extralaryngeal structures.

functional abduction of the vocal fold, and if so, 2) to determine if vocal fold abduction could be electrically paced with the inspiratory phase of respiration by means of an external circuit.

METHODS

The subject in the study was a 16-year-old boy with a right vocal fold paralysis following resection of a carotid body tumor 5 months previously. Surgery was performed under local anesthesia with intravenous sedation. The thyroplasty procedure employed a refinement of the original technique of Isshiki et al.[10] Prior to the surgery, a plethysmographic transducer belt (Pneumotrace) was placed around the patient's chest to monitor inspiratory effort. A flexible nasopharyngoscope was positioned above the larynx to record spontaneous and evoked vocal fold movement videographically. A horizontal incision was made in the anterior neck. The thyroid ala was exposed and rotated to provide easy access for stimulation of the PCA muscle on the paralyzed side. A 22-gauge Teflon-insulated hypodermic needle with exposed tip (TECA) served as the stimulating (cathode) electrode. The needle was inserted along the posterior border of the thyroid lamina to target the middle portion of the laryngeal abductor muscle. A second TECA needle placed in skin over the neck midline served as the reference (anode) electrode.

The external apparatus used to pace the PCA muscle is shown in Fig 1. The system combined conventional equipment and common circuit elements in the following sequence: respiratory belt transducer, bridge, amplifier, gate, stimulator, patient stimulus isolation unit, and stimulus electrodes. Steady state voltages arising from stretch of the transducer around the chest were nulled out by the bridge. Respiratory signals generated during chest expansion were then amplified and filtered by the amplifier. These signals were fed into a threshold detector or gate that established the length of time that the stimulus would be turned on during inspiration. The parameters of the gated stimulus were controlled by the stimulator. The output stimuli were delivered to the electrodes through an isolation unit. With this design, stimuli triggered and gated by the respiratory cycle could be modulated to effectively pace contraction of the PCA muscle with each inspiration.

Prior to pacing, the system was used to locate the PCA muscle. The stimulator was set to continuously deliver pulses at a frequency of 1 Hz. A low-frequency stimulus was chosen in order to avoid changes in heart rate that could result from inadvertent activation of the vagus. Twitch responses of the larynx on the paralyzed side were observed on the video monitor during advance of the cathode electrode. These responses could be attributed to nonspecific activation of structures external to the larynx, such as the internal laryngeal nerve or strap muscles. When the electrode tip found the PCA muscle, isolated abductive twitches of the paralyzed vocal fold were observed.

System parameters were then adjusted to generate a PCA contraction effecting a smooth vocal fold abduction. This was achieved with a stimulus train of 1.5 seconds at a tetanizing frequency of 30 Hz. The pulse duration was set at 2 milliseconds to optimally activate denervated muscle fibers, as previously determined in a canine study.[8] Stimulus intensities ranging from 1 to 10 mA were explored to assess changes in magnitude of abduction.

The utility of the system as a laryngeal pacemaker was investigated. The stimulator was returned to the control of gated signals derived from the respiratory transducer. Circuit parameters were adjusted to determine if abduction of the paralyzed vocal fold could be synchronized with inspiration. Finally, the capacity of laryngeal pacing to achieve functional abduction was evaluated by comparing the evoked vocal fold motion to spontaneous movement on the normal side.

RESULTS

Endoscopic videographs of the patient's vocal folds are shown in Fig 2. Figure 2A shows the vocal folds at rest. During phonation, the left vocal fold adducted to approximate the paralyzed fold (Fig 2B). Upon deep inspiration, the left vocal fold abducted beyond the rest position (Fig 2C). In contrast, there was an absence of associated movement of the right vocal fold during these behaviors, indicating a complete unilateral laryngeal paralysis.

After the cathode electrode had been positioned within the PCA muscle, the system parameters were optimally adjusted to evoke a smooth abduction. The relationship between stimulus intensity and vocal fold displacement was investigated. Amplitudes from 1.5 to 7 mA covered the entire dynamic range of responses from threshold to maximum.

Figure 3 shows a sequence of endoscopic images captured at various time points during paced stimulation of the PCA muscle. In the lower right corner of each frame, oscillographic tracings from the respiratory sensor (upper trace), gate (middle trace), and stimulator (lower trace) are shown over the course of three respiratory cycles. In the respiratory recording, the onset of an inspiration was denoted by an upward deflection. The gate was set for activation at a relatively low threshold, such that stimuli were delivered over a considerable portion of each inspiratory phase. The arrow in each frame indicates the time point in the pacing cycle at which the endoscopic image was obtained. The series of videographs in Fig 3A-C illustrate opening of the glottis during the onset of paced stimulation, while Fig 3D-F shows closing of the glottis during the offset of a pacing cycle. The timing of evoked abduction was consistently synchronized with inspiration, owing to the fact that stimulation was reproducibly gated with respiration.

The magnitude of abduction generated by paced stimulation could be appreciated by the progressive lateral displacement of the arytenoid in each still frame (Fig 3A-C). The extent of right-sided abduction was impressive. In fact, the increase in glottal area could be largely attributed to right-sided abduction, in view of the nominal level of spontaneous movement on the contralateral side. Finally, the level of paced abduction was clearly functional when compared to left-sided abduction during maximum inspiratory effort (Fig 1C). At the end of a pacing cycle, the vocal fold returned to the midline (Fig 3D-F) to allow for phonation and airway protection. This attested to the physiologic nature of electrical pacing, in that glottal opening was only induced during inspiration, to provide for ventilation.

DISCUSSION

This investigation represents the first report of electrical pacing in the human larynx. By means of a simple system, paced vocal fold abduction in synchrony with inspiration was successfully demonstrated. The magnitude of abduction generated was functional, and sufficient to improve ventilation. The stimulus parameters previously identified as appropriate and complementary for activation of denervated PCA muscle in the canine[8] were found effective in activating human laryngeal muscle.

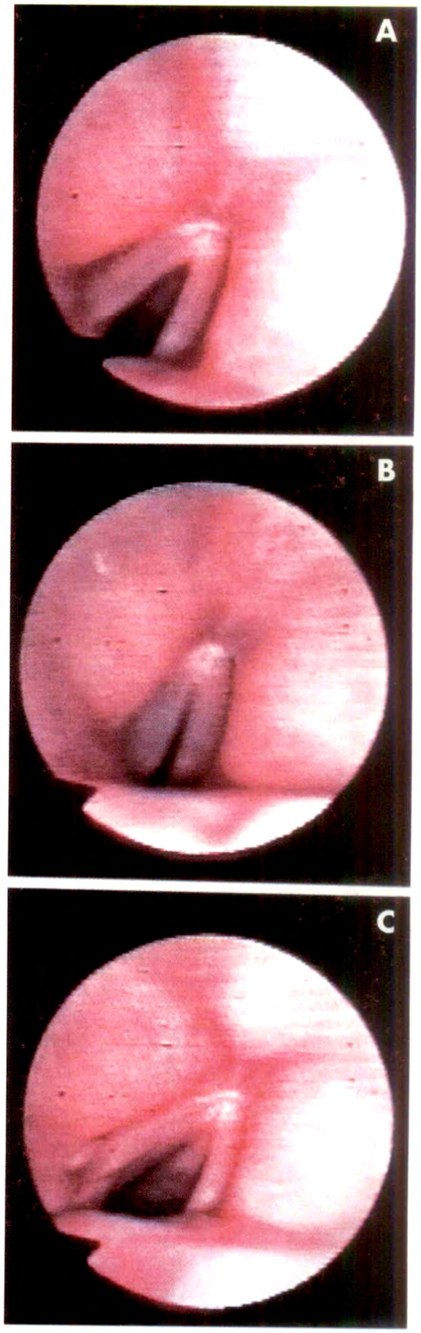

Fig 2. Endoscopic videographs of patient with unilateral laryngeal paralysis. Glottis is shown A) at rest, B) during phonation, and C) upon maximal inspiratory effort. Right vocal fold is paralyzed in paramedian position and does not participate in abduction or adduction.

This study is an initial step toward clinical investigation of FNS treatment for laryngeal paralysis. As presented in the paper, a patient with unilateral paralysis is an ideal model for preliminary evaluation of laryngeal pacing. Surgical exposure of the larynx during thyroplasty allows controlled insertion of electrodes for external pacing while the patient is under

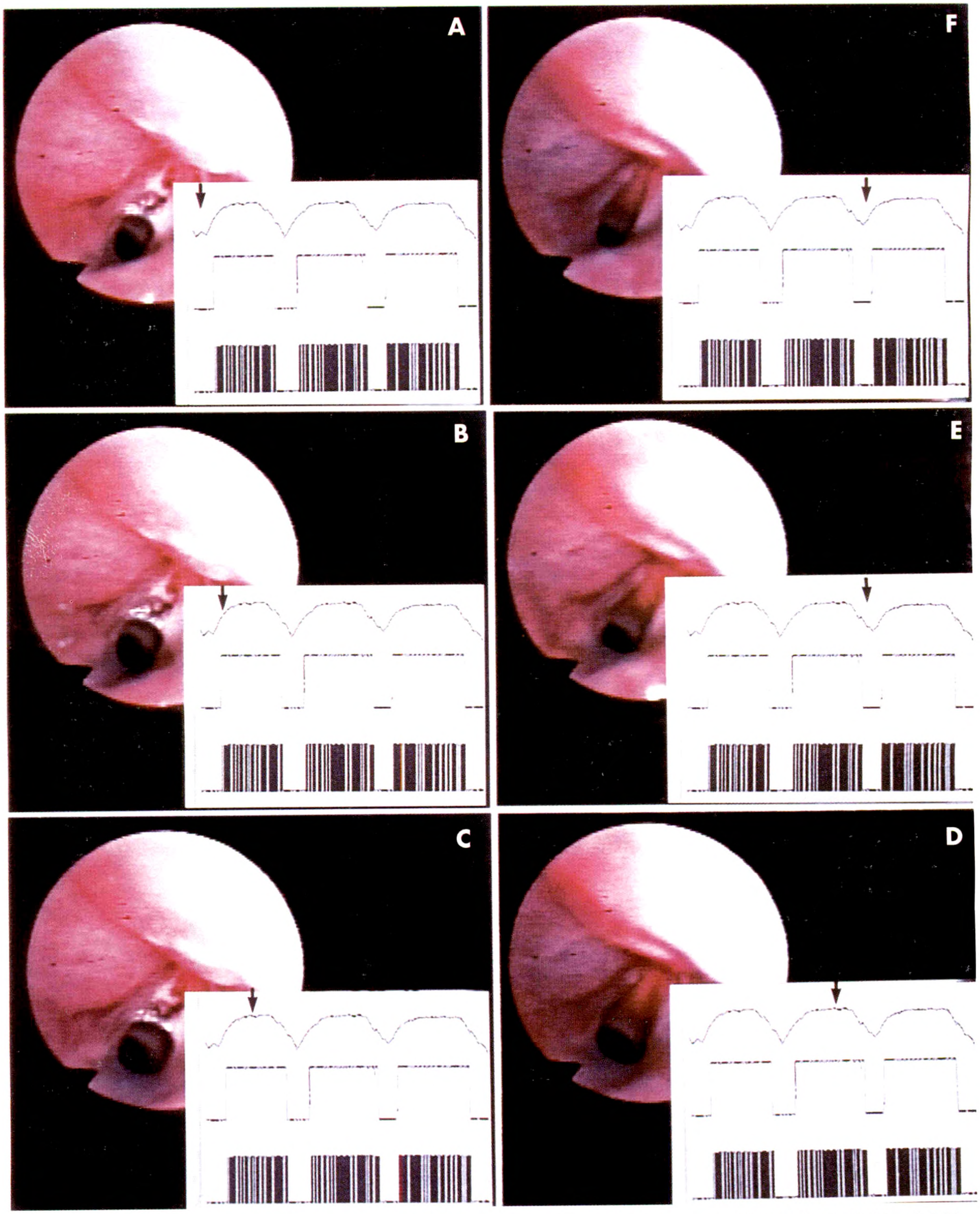

Fig 3. Endoscopic images during paced stimulation of paralyzed posterior cricoarytenoid muscle. A-C) Vocal fold abduction during onset of pacing. D-F) Passive return of vocal fold to midline with offset of stimulation. Oscillographic recordings from respiratory transducer, gate, and stimulator appear in right corner of each frame. Stimuli were delivered consistently at 30 Hz, but pulse trains appeared irregular because of aliasing by digital storage oscilloscope at low sweep speeds. Arrow — time point in pacing cycle at which endoscopic image was captured.

local anesthesia and sedated. Although the model is unilateral paralysis, the primary clinical focus of this technology is to restore glottal opening in the bilaterally paralyzed patient. An external approach would provide a means for screening patients who are candidates for pacemaker implantation. Specifically, patients with poor stimulated abduction would be excluded from surgery by this noninvasive test.

In addition to serving as a diagnostic tool, external electrical pacing may have direct therapeutic value. In canine studies, chronic stimulation of denervated PCA muscles prevented muscle fiber atrophy and enhanced contractile strength (Zealear et al, unpublished observations). Further, in cases in which nerve regeneration was allowed, FNS appeared to influence the pattern of reinnervation in a beneficial way (Zealear et al, unpublished observations). In particular, selective reinnervation by intrinsic nerve fibers was encouraged, while reconnection by foreign fibers was repressed. Remarkably, these effects were produced with application of FNS over a period of only 3 to 5 weeks. In the patient, repeated clinical application of external pacing in electrotherapeutic sessions may maintain muscle mass and promote appropriate reinnervation, thereby avoiding synkinesis.

Finally, external pacing may serve in a functional capacity to provide the patient a means of dynamic ventilation while awaiting reinnervation. The apparatus could function as an assistive device during physical exertion, or at night to prevent sleep apnea. An external pacemaker would have advantages over an implantable one in this instance. A multielectrode array or microstimulator would be the only invasive component and could be introduced through the TECA stimulating needle.[11] The electrode array could be activated transcutaneously by a radio frequency or current induction means. Both physician and patient would have complete access to pacing components for parameter adjustment. Such a portable external pacing system could be used on a short- or long-term basis, depending on the needs of the patient.

If employed as a diagnostic screening tool, as a clinical therapy, and as an assistive technology during reinnervation, external laryngeal pacing may provide a means for complete rehabilitation of the paralyzed larynx without need for further intervention.

REFERENCES

1. Zealear DL, Dedo HH. Control of paralyzed axial muscles by electrical stimulation. Acta Otolaryngol (Stockh) 1977;83:514-27.

2. Obert PM, Young KA, Tobey DN. Use of direct posterior cricoarytenoid stimulation in laryngeal paralysis. Arch Otolaryngol 1984;110:88-92.

3. Bergmann K, Warzel H, Eckhardt H-U, Gerhardt H-J. Respiratory rhythmically regulated electrical stimulation of paralyzed laryngeal muscles. Laryngoscope 1984;94:1376-80.

4. Zealear DL, Herzon GD. Progress toward the development of a chronically implantable laryngeal pacemaker. In: Fujimura O, ed. Vocal physiology: voice production, mechanisms and functions. New York, NY: Raven Press, 1988:443-8.

5. Kim KM, Choi HS, Kim GR, Hong WP, Chun YM, Park YJ. Laryngeal pacemaker using a temperature sensor in the canine. Laryngoscope 1987;97:1207-10.

6. Otto RA, Temper J, Davis W, Homeyer D, Stroble M. Coordinated electrical pacing of vocal cord abductors in recurrent laryngeal nerve paralysis. Otolaryngol Head Neck Surg 1985;93:634-8.

7. Sanders I. Electrical stimulation of laryngeal muscle. Otolaryngol Clin North Am 1991;24:1253-74.

8. Zealear DL, Rainey CL, Jerles ML, Tanabe T, Herzon GD. Technical approach for reanimation of the chronically denervated larynx by means of functional electrical stimulation. Ann Otol Rhinol Laryngol 1994;103:705-12.

9. Zrunek M, Streinzer W, Mayr W, Burian K, Thoma H, Gruber H. Experiments of acute and chronic direct electrical stimulation of glottis opening muscles. Eur Arch Otorhinolaryngol 1991;248:445-8.

10. Isshiki N, Morita H, Okamura H, Hiramoto M. Thyroplasty as a new phonosurgical technique. Acta Otolaryngol (Stockh) 1974;78:451-7.

11. Loeb GE, Zamin CJ, Schulman JH, Troyk PR. Injectable microstimulator for functional electrical stimulation. Med Biol Eng Comput 1991;29:NS13-NS19.

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ROLE OF THE AGGER NASI CELL IN CHRONIC FRONTAL SINUSITIS

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Agger nasi cells contribute to nasofrontal duct (NFD) obstruction and chronic frontal sinus disease. To investigate this relationship, we conducted a review of the surgical outcome and computed tomographic imaging in 26 patients with chronic frontal sinusitis. Coronal and sagittal images were used to delineate the anatomic variability and mucosal disease in the NFD and frontal sinus region. Data from coronal and sagittal images were compared. The results were also correlated with the outcome of frontal sinus surgery in patients with a clinical history of chronic frontal sinus disease. Our data suggest that agger nasi cell pneumatization with narrowing of the frontal sinus outflow tract is a significant cause of persistent frontoethmoid pain and chronic frontal sinusitis. Sagittal reformatted images are more capable than coronal images of demonstrating agger nasi cell encroachment on the NFD, as well as NFD mucosal disease. Endoscopic frontal sinusotomy is an effective treatment for chronic frontal sinus disease.

KEY WORDS — agger nasi cell, frontal sinus, frontal sinusitis, nasofrontal duct.

INTRODUCTION

Chronic frontal sinusitis presents a challenging diagnostic problem for the otorhinolaryngologist. Frontoethmoid pain may be the solitary presenting symptom. Pathologic findings on nasal endoscopy are often absent. Computed tomography (CT) scanning in the coronal plane with sagittal reformatted images may demonstrate frontal sinus mucosal disease, as well as frontal sinus outflow tract obstruction.

Endoscopic frontal sinusotomy permits inspection of the frontal sinus and frontal recess, and the removal of obstructing anterior ethmoid cells and diseased mucosa, restoring frontal sinus drainage.[1-3] Schaefer and Close[1] found in their series of 36 patients that the most appropriate candidates for endoscopic frontal sinusotomy were those with either frontal recess or anterior ethmoid obstructive disease, as well as those with diseased mucosa or mucoceles in the medial aspect of the frontal sinus.

The agger nasi is a small crest of bone located in the lateral nasal wall just anterior and superior to the anterior attachment of the middle turbinate. Pneumatization of the agger nasi bone creates an air cell, the agger nasi cell, that may expand sufficiently to encroach upon the medial aspect of the frontal sinus floor, narrowing or obstructing the nasofrontal duct (NFD; Fig 1).[4-7] Therefore, the agger nasi cell has been implicated in the pathogenesis of persistent frontoethmoid sinus pain and chronic frontal sinusitis.

To investigate the role of the agger nasi cell as a cause of NFD obstruction and chronic frontal sinus disease, we studied the relationship of the NFD and frontal recess to the agger nasi cell with the use of CT in the coronal plane along with sagittal reformatted images. The outcome of medical and surgical management, including endoscopic frontal sinusotomy with removal of agger nasi cells, is reviewed and correlated with findings from CT scan images.

METHODS

The medical records and surgical findings of 26 patients who presented with a clinical history of chronic frontal sinusitis were retrospectively reviewed. Noncontrast coronal CT scans of the paranasal sinuses were available for review in 24 patients. All CT examinations included contiguous 3-mm-

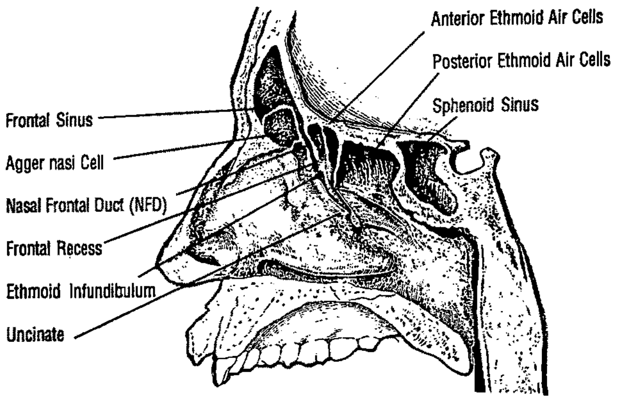

Fig 1. Agger nasi cell and its relationship to nasofrontal region.

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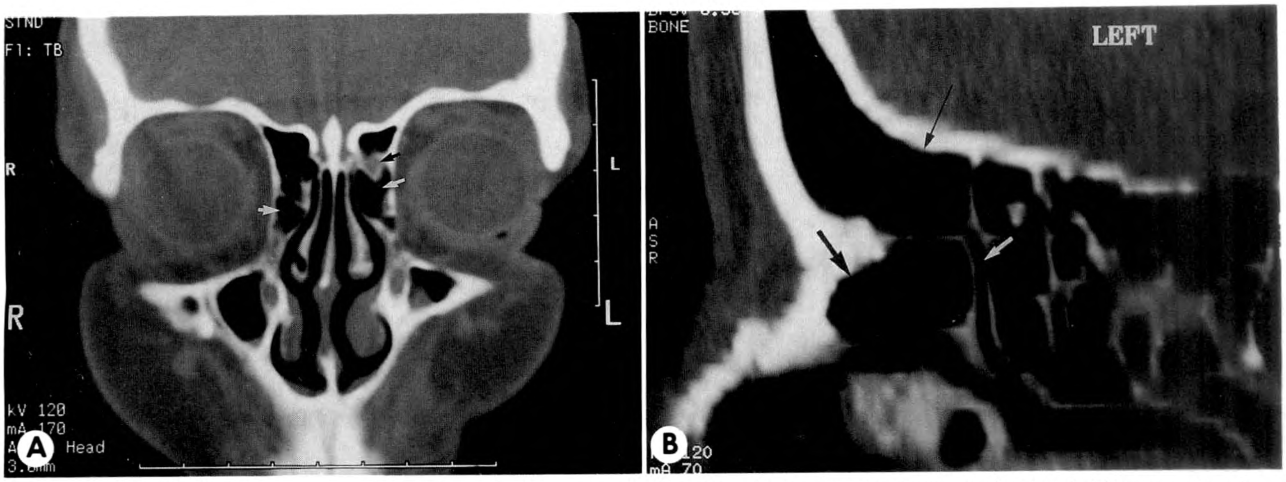

Fig 2. (Case 1) A) Coronal computed tomography (CT) scan shows bilateral agger nasi cell pneumatization (white arrows) and mucosal disease in region of nasofrontal duct (black arrow). B) Sagittal reformatted image shows frontal sinus (thin black arrow) and agger nasi cell (thick black arrow) encroaching on nasofrontal duct (white arrow).

thick coronal sections through the paranasal sinuses angled perpendicular to the hard palate. Supplemental contiguous 1.5-mm-thick axial sections through the frontal sinuses and frontal sinus outflow tracts were obtained in 4 patients. Contiguous reformatted sagittal images through the NFD using 1-mm-thick sections were obtained in 8 patients. The sagittal images were derived either from the coronal images (4 patients) or from the axial images (4 patients).

Patients were divided into two groups: those who were treated surgically (21 patients) and those who responded to medical therapy (5 patients). The coronal CT scans of 28 frontal sinuses that were treated surgically (surgical frontal sinuses [SFSs]) were compared to the coronal CT scans of 20 frontal sinuses that were not treated surgically (nonsurgical frontal sinuses [NSFSs]). Included in the NSFS group were the sinuses in patients who responded to medical therapy, as well as the nonsurgical sinus in patients who underwent unilateral surgery. Coronal CT scans in 11 of the sinuses in the SFS group and 2 of the sinuses in the NSFS group showed evidence of previous surgery. Evidence of previous sinus surgery in sagittal reformatted images was present in 4 of the sinuses in the SFS group and in 2 of the sinuses in the NSFS group.

The CT images were interpreted by a single observer without knowledge of the clinical history. The coronal images were analyzed for the presence of frontal sinus mucosal disease, anterior ethmoid mucosal disease, NFD or frontal recess mucosal disease, pneumatization of the agger nasi, and agger nasi cell encroachment upon the NFD or frontal recess.

Sagittal images were assessed for the presence of NFD or frontal recess mucosal disease and agger nasi cell encroachment on the NFD or frontal recess. Data from coronal images and sagittal reformatted images were then compared.

The majority of patients treated surgically underwent endoscopic ethmoidectomy with frontal sinusotomy and removal of agger nasi cells. While the surgical procedures vary slightly as dictated by the individual's anatomy, the standard surgical technique is as follows. After the infundibulotomy, the agger nasi cell is visualized endoscopically with a 0° telescope. If this cell is significantly pneumatized, it may be separated from the lateral surface of the middle turbinate by infracturing its anterior and medial wall. The agger cell wall is resected with upbiting forceps to reveal the agger nasi cell dome. A 30° or 70° scope is now used for visualization. At this point, care should be exercised to differentiate a superiorly placed dome from the frontal recess. A probe or frontal curette is utilized to palpate the middle turbinate and posterior wall of this dome. If a separate cell wall is noted, up-biting forceps or a frontal giraffe forceps is employed to resect these remnants until the frontal ostium is clearly identified. This technique essentially marsupializes an expanded frontal recess because of the presence of this agger nasi cell. Instrument trauma to the mucosa of the frontal recess and ostium must be avoided, since scar tissue may form, creating secondary NFD stenosis.

The outcome of frontal sinus surgery was evaluated by patient report of the resolution of preoperative symptoms, and findings on postoperative nasal endoscopy. These surgical results were evaluated and correlated with preoperative CT scan findings. All surgical management and postoperative care was performed by the senior author (J.B.J.).

CASE REPORTS

Case 1. A 37-year-old woman experienced recur-

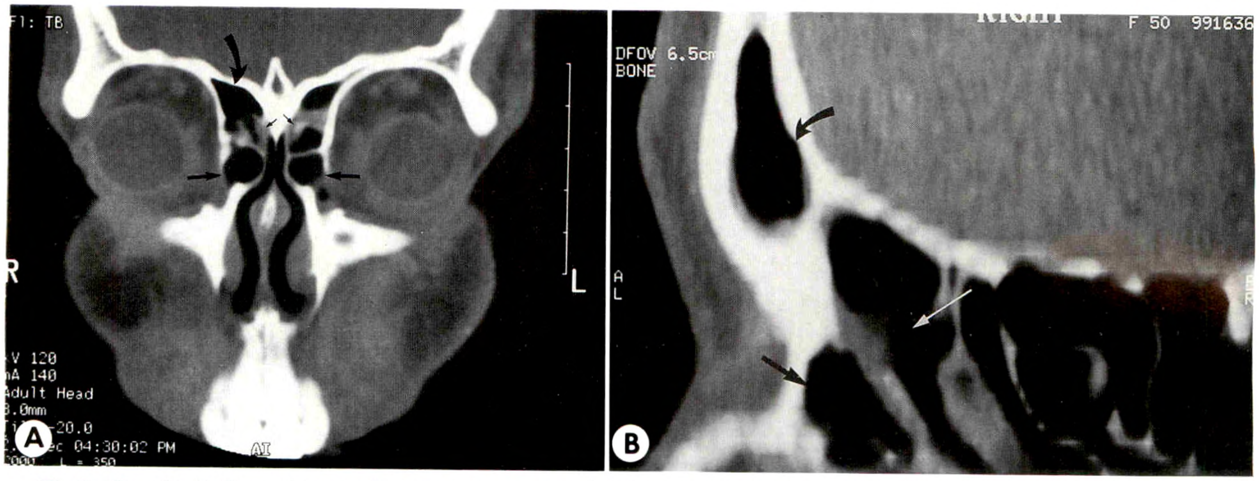

Fig 3. (Case 2) A) Coronal image demonstrates agger nasi cell pneumatization (larger black arrows) and mucosal disease in frontal sinus (curved black arrow) and nasofrontal duct region (small black arrows). B) Sagittal image shows enlarged agger nasi cell (straight black arrow) and mucosal disease in frontal sinus (curved black arrow) and in nasofrontal duct (white arrow).

rent bilateral frontoethmoid headache and rhinorrhea despite aggressive medical management with antibiotics, steroids, and antihistamines. Nasal endoscopy revealed enlarged agger nasi cells bilaterally, along with frontal recess mucosal edema and middle turbinate deformities. Coronal CT scans demonstrated agger nasi cell pneumatization bilaterally, with soft tissue densities in the anterior ethmoid drainage pathways (Fig 2A). Sagittal reformatted images of the nasofrontal region illustrated exuberant pneumatization of the agger nasi cells bilaterally, with encroachment upon the frontal recess and nasofrontal duct (Fig 2B). The patient underwent bilateral endoscopic frontal sinusotomies with removal of obstructing agger nasi cells and mucosal disease. She has remained asymptomatic since the time of her surgery 11 months ago.

Case 2. A 50-year-old woman had experienced four or five episodes of frontal sinusitis per year for the past 10 years. Her symptoms included frontoethmoid headaches, facial pain, rhinorrhea, and nasal obstruction. The episodes of acute frontal sinusitis recurred despite adequate treatment with antibiotics. Endoscopic examination showed frontal recess mucosal edema. Agger nasi cell pneumatization was demonstrated bilaterally on coronal CT scan images (Fig 3A). The agger nasi cells encroached on the frontal recesses and anterior ethmoid cell complexes.

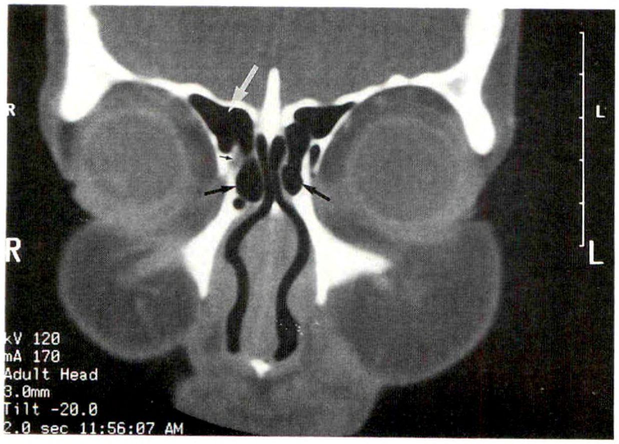

Fig 4. (Case 3) Coronal image reveals enlarged agger nasi cell (larger black arrows) and mucosal disease in frontal recess region (small black arrow) and frontal sinus (white arrow).

Sagittal reformatted images confirmed the presence of mucosal disease in the NFDs and frontal recesses (Fig 3B). Endoscopic frontal sinusotomies with removal of the agger nasi cell dome were performed. Postoperative nasal endoscopic examination revealed patent nasofrontal ducts. The patient has been asymptomatic since her surgery 1 year ago.

Case 3. A 33-year-old man reported a long history of recurrent bilateral frontoethmoid pain, with at least 10 episodes of frontal sinusitis per year. He had no response to prolonged courses of steroids, antibiotics, and nasal steroid sprays. Frontal recess and middle meatus edema were found on nasal endoscopic examination. The CT scan images revealed enlarged agger nasi cells with encroachment on the frontal recess (Fig 4). Resultant mucosal disease was depicted in the frontal sinus, NFD, and anterior ethmoid cell complex. The patient underwent bilateral endoscopic frontal sinusotomies with removal of obstructing agger nasi cells. The patient remains asymptomatic 1 year postoperatively.

RESULTS

Patient Background. From May 1990 to March 1994, 26 patients (13 men, 13 women) were evaluated and treated for chronic frontal sinusitis. The average age of the patients was 43 years, with a range

TABLE 1. PATIENT BACKGROUND

	No. of Patients (N = 26)
No. of male patients	13
Frontoethmoid mucocele	4
Nasal polyposis	2
Previous sinus surgery (range, 1 to 4 procedures per patient)	10
Previous frontal sinus surgery	2
Type of previous surgery	
Unilateral endoscopic ethmoidectomy	7
Bilateral endoscopic ethmoidectomy	4
Caldwell-Luc procedure	2
Endoscopic frontal sinusotomy	1
External frontoethmoidectomy	1
Septoplasty with turbinectomies	1

Mean age of patients was 43 years (range, 18 to 74 years).

TABLE 2. SYMPTOMS AND PHYSICAL FINDINGS

	Patients (N = 26)	
	No.	*%*
Symptoms		
Frontoethmoid pain and/or pressure	25	96
Generalized facial pain and/or pressure	21	81
Nasal obstruction	13	50
Generalized headache	12	46
Rhinorrhea	8	31
Periorbital swelling	5	19
Blurred vision	3	12
Physical findings		
Polypoid nasal mucosa	20	77
Frontal recess edema	18	69
Middle meatus disease	9	35
Septal deviation	8	31
Frontal sinus tenderness	5	19
Frontal recess stenosis	2	8
Nasal polyps	2	8
Nasofrontal duct edema	2	8
Periorbital edema	2	8
Synechiae	2	8
Globe displacement	1	4
Middle meatus purulence	1	4
Proptosis	1	4
Frontal recess purulence	0	0

from 18 to 72 years. Four patients had frontoethmoid mucoceles, and 2 patients had extensive nasal polyposis. Ten of the 26 patients had undergone previous sinus surgery not involving the frontal sinus, while an additional 2 patients had previous frontal sinus surgery (Table 1).

The predominant symptoms (Table 2) in patients with chronic frontal sinusitis included frontoethmoid pain and/or pressure (96%) and generalized facial pain and/or pressure (81%). Other notable symptoms were nasal obstruction (50%), generalized headaches (46%), rhinorrhea (31%), periorbital swelling (19%), and blurred vision (11%). Blurred vision and periorbital swelling occurred most commonly in the presence of a frontoethmoid mucocele. The classic sinusitis symptom triad of sinus pain, nasal obstruction, and rhinorrhea only occurred in 19% (5/26) of patients. Nasal endoscopy (Table 2) revealed frontal recess mucosal edema in 69% of patients and frontal recess stenosis in 7% of patients. Nonspecific findings included polypoid nasal mucosa (77%) and septal deviation (31%). Periorbital edema, globe displacement, and proptosis were found most frequently in patients with frontoethmoid mucocele. Purulent discharge from the middle meatus was only found in 1 of the 26 patients. No patient had evidence of frontal recess purulence.

Management of Chronic Frontal Sinus Disease. Chronic frontal sinusitis resolved with aggressive medical management (antibiotics, local decongestion, and topical steroid sprays) in 5 patients (19%). Twenty-one of the 26 patients (81%) had symptoms refractory to medical management and were treated with surgery (Table 3). Eighty-six percent of patients (18/21) underwent endoscopic frontal sinusotomy with anterior endoscopic ethmoidectomies and removal of any agger nasi cells. This procedure was performed unilaterally in 11 patients and bilaterally in 7 patients. Placement of a frontonasal silicone catheter was needed in 2 of these patients to ensure sufficient drainage and ventilation of the frontal sinus. Two patients required combined external and endoscopic approaches. External ethmoidectomy alone was warranted in 1 patient.

Fifteen of the 18 patients (83%) who underwent endoscopic frontal sinusotomy experienced either complete resolution or significant improvement of their frontoethmoid pain. Postoperative nasal endoscopy revealed patent NFDs. Two of the 3 patients who required either combined endoscopic frontal sinusotomy with an external frontal sinus approach or external frontoethmoidectomy reported resolution of their preoperative symptoms.

Chronic frontal sinusitis persisted in 19% (4/21) of patients treated surgically. The continuation of frontal sinus disease occurred after endoscopic frontal sinusotomy in 3 of the patients and after endoscopic frontal sinusotomy with external frontal sinusotomy in a fourth patient. Frontal recess stenosis and/or NFD stenosis were found in 2 of the patients. Anomalous drainage of the NFD and NFD obstruction by an enlarged supraorbital ethmoid cell occurred in the third and fourth patients, respectively. All 4 patients

TABLE 3. MANAGEMENT OF CHRONIC FRONTAL SINUSITIS

		No. of Patients
Nonsurgical or medical management		5
Surgical management		21
Unilateral frontal sinusotomy, agger nasi cell dome removal	11	
Bilateral frontal sinusotomy, agger nasi cell dome removal	7	
Combined endoscopic frontal sinusotomy with external frontoethmoidectomy	1	
Combined endoscopic frontal sinusotomy with external frontal sinus trephination	1	
External frontoethmoidectomy	1	

required further surgical intervention.

Computed Tomography Analysis. Analysis of coronal images (Table 4) revealed frontal sinus disease in 82% of SFSs and 25% of NSFSs. Anterior ethmoid sinus disease was found in 93% of SFSs and in 40% of NSFSs. Disease in the NFD or frontal recess was demonstrated in 93% of SFSs and 65% of NSFSs. Agger nasi cell pneumatization was found in 54% of SFSs and 70% of NSFSs. Encroachment of the agger nasi cell on the frontal recess or NFD was detected in 43% of SFSs and 50% of NSFSs.

Analysis of coronal CT scans in patients with sagittal reformatted images (Table 5) demonstrated frontal recess or NFD mucosal disease in 54% of SFSs and 60% of NSFSs. In coronal images, agger nasi cell encroachment on the frontal recess or NFD was found in 45% of SFSs and 20% of NSFSs. Evaluation of sagittal images revealed frontal recess or NFD mucosal disease in 91% of SFSs and 20% of NSFSs. Agger nasi cell encroachment on the frontal recess or NFD was found in 82% of SFSs and 20% of NSFSs in sagittal reformatted images.

TABLE 4. CORONAL COMPUTED TOMOGRAPHY FINDINGS IN PATIENTS WITH FRONTAL SINUS DISEASE (24 PATIENTS, 48 SINUSES)

	Surgical Sinuses (N = 28)*		*Nonsurgical Sinuses† (N = 20)*	
Anatomic Location	*No.*	*%*	*No.*	*%*
Frontal sinus mucosal disease	23	82	5	25
Anterior ethmoid mucosal disease	26	93	8	40
Frontal recess and/or NFD mucosal disease	26	93	13	65
Agger cell (AC) pneumatization	15	54	14	70
AC encroaching on NFD and/or frontal recess	12	43	10	50

NFD — nasofrontal duct.

*Eleven sinuses had previous surgery.

†Two sinuses had previous surgery.

TABLE 5. CORONAL VERSUS SAGITTAL COMPUTED TOMOGRAPY FINDINGS (8 PATIENTS, 16 SINUSES)

	Surgical Sinuses (N = 11)*				*Nonsurgical Sinuses† (N = 5)*			
	Sagittal		*Coronal*		*Sagittal*		*Coronal*	
Anatomic Location	*No.*	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
Frontal recess or NFD mucosal disease	10	91	6	54	1	20	3	60
Agger nasi cell encroaching on NFD or frontal recess	9	82	5	45	1	20	1	20

NFD — nasofrontal duct.

*Four sinuses had previous surgery.

†Two sinuses had previous surgery.

In comparison to coronal CT scans, sagittal reformatted images (Table 5) demonstrated frontal recess or NFD mucosal disease in 37% more SFSs. Agger nasi cell encroachment on the NFD or frontal recess, in sagittal images, was present in 37% more SFSs than in coronal images. There was no difference in the presence of agger nasi cell encroachment on the frontal recess or NFD when coronal and sagittal images were compared in NSFSs.

Surgical outcome after endoscopic frontal sinusotomy was then correlated with preoperative CT scan findings (Table 6). Fifteen of 18 patients had improvement or complete resolution of their symptoms after endoscopic frontal sinusotomy.

In this group of patients, coronal images revealed frontal recess or NFD mucosal disease in 71%, agger nasi cell pneumatization in 78%, and agger nasi cell encroachment on the frontal recess or NFD in 64% of the patients. Sagittal reformatted images in these patients revealed frontal recess or NFD mucosal disease in 100% of the patients. Agger nasi cell

TABLE 6. COMPUTED TOMOGRAPHY FINDINGS IN PATIENTS WITH IMPROVED SYMPTOMS AFTER FRONTAL SINUSOTOMY

	Coronal		*Sagittal*	
Anatomic Location	*No.*	*%*	*No.*	*%*
Agger nasi cell encroaching on NFD and/or frontal recess	9	64	5	83
NFD or frontal recess mucosal disease	10	71	6	100

Total 15 patients, 14 coronal images, 6 sagittal images available.

encroachment on the frontal recess or NFD was present in 83% of the patients.

DISCUSSION

The embryologic frontal furrows or pits (outgrowths of the middle meatus in the frontal recess region) evaginate and pneumatize the frontal and anterior ethmoid regions in a variable pattern.[5] The agger nasi cell develops from the first frontal pit and communicates with the middle meatus medial to the uncinate process. The second frontal pit extends as an anterior ethmoid air cell to pneumatize the frontal region as the frontal sinus. The third and fourth frontal pits develop into anterior ethmoid cells that communicate with the middle meatus superior to the semilunar hiatus of the ethmoid infundibulum. The frontal sinus may develop by direct extension of the frontal recess or by extension of an anterior ethmoid cell. In the latter case, the NFD may be tortuous because of the impingement of neighboring anterior ethmoid cells, including the agger nasi cell.

The agger nasi cell is the most constant and anterior of the anterior ethmoid air cells.[4] The prevalence and location of the agger nasi cell varies in differing studies. In 1942, Van Alyea[4] reported its presence in 89% of specimens. More recently, Bolger et al[8] encountered the agger nasi cell in 98.5% of patients. The agger nasi cell is bordered by the frontal process of the maxilla anteriorly, by the frontal recess and sinus superiorly, by the nasal bones anterolaterally, by the lacrimal bone inferolaterally, by the uncinate process inferomedially, and by the ethmoid infundibulum posteriorly.[2,8,9] Stammberger and Wolf[7] and Kuhn et al[9] described its location as anterior and superior to the insertion of the middle turbinate on the lateral nasal wall. Kennedy and Zinreich[10] stated that the agger nasi cell is located below the frontal sinus, extending to the frontal recess superiorly and to the lacrimal fossa inferolaterally. The agger nasi cell has also been described as originating from the anterior superior aspect of the infundibular groove.[4,8] These variations in description may be secondary to the differing degrees of pneumatization of the agger nasi cell in conjunction with its complex relationship to the frontal, maxillary, lacrimal, and nasal bones. The agger nasi cell may pneumatize into the frontal recess and cause frontal sinus outflow tract obstruction, which leads to frontoethmoid pain and frontal sinus disease.[9]

Because of the protean nature of the symptoms in chronic frontal sinusitis, the otorhinolaryngologist must have a high index of suspicion to make the appropriate clinical diagnosis. Frontoethmoid sinus pain may be the only evidence to indicate the presence of chronic frontal sinusitis. In this series, the classic symptoms of sinusitis (sinus pain, nasal obstruction, and rhinorrhea) occurred in fewer than 20% of patients with chronic frontal sinusitis. Although frontal recess mucosal edema was detected on nasal endoscopy in the majority of cases, the absence of findings on nasal endoscopy does not rule out the presence of anterior ethmoid and frontal sinus disease.

In evaluating agger nasi cell encroachment on the NFD as a cause of chronic frontal sinusitis, sagittal reformatted images more clearly define this specific anatomic relationship. In addition, frontal recess or NFD mucosal disease is more clearly delineated. These radiologic findings correlated with anatomic relationships encountered during endoscopic frontal sinusotomies. Sagittal reformatted images provide the surgeon with valuable information concerning the course of the NFD and the nature of frontal sinus outflow tract disease.

When sagittal reformatted images on patients who underwent surgery were reviewed, 82% demonstrated agger nasi cell encroachment on the NFD or frontal recess. This result suggests that agger nasi cell encroachment is a significant etiologic mechanism for chronic frontal sinusitis. Endoscopic frontal sinusotomy is an effective surgical procedure for the treatment of chronic frontal sinusitis secondary to NFD obstruction. In this study, endoscopic frontal sinusotomy was effective in relieving frontoethmoid pain in 83% of patients.

CONCLUSION

Agger nasi cell pneumatization with narrowing of the frontal sinus outflow tract is a significant cause of persistent frontoethmoid pain and chronic frontal sinusitis. Sagittal reformatted images, in comparison to coronal plane images, are superior in demonstrating agger nasi cell encroachment on the NFD, as well as NFD mucosal disease. Sagittal images are important in preoperative planning to define the course of the NFD. Endoscopic frontal sinusotomy is an effective treatment for chronic frontal sinus disease.

REFERENCES

1. Schaefer SD, Close LG. Endoscopic management of frontal sinus disease. Laryngoscope 1990;100:155-60.

2. Loury MC. Endoscopic frontal recess and frontal sinus ostium dissection. Laryngoscope 1993;103:455-8.

3. Metson R. Endoscopic treatment of frontal sinusitis. Laryngoscope 1992;102:712-6.

4. Van Alyea OE. Nasal sinuses: an anatomic and clinical consideration. Baltimore, Md: Waverly Press, 1942:66-140.

5. Schaeffer JP. The embryology, development and anatomy of the nose, paranasal sinuses, nasolacrimal passageways and olfactory organ in man. York, Pa: Maple Press, 1920:50-105.

6. Kennedy DW, Josephson JS, Zinreich SJ, Mattox DE, Goldsmith MM. Endoscopic sinus surgery for mucoceles: a viable alternative. Laryngoscope 1989;99:885-95.

7. Stammberger H, Wolf G. Headaches and sinus disease: the endoscopic approach. Ann Otol Rhinol Laryngol 1988;97 (suppl 134).

8. Bolger WE, Butzin CA, Parsons DS. Paranasal sinus bony anatomic variations and mucosal abnormalities: CT analysis for endoscopic sinus surgery. Laryngoscope 1991;101:56-64.

9. Kuhn FA, Bolger WE, Tisdale RG. The agger nasi cell in frontal recess obstruction: an anatomic, radiologic and clinical correlation. Operative Techniques Otolaryngol Head Neck Surg 1991;2:226-31.

10. Kennedy DW, Zinreich SJ. The functional endoscopic approach to inflammatory sinus disease: current perspectives and technique modifications. Am J Rhinol 1988;2:89-96.

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DIZZINESS AND BALANCE DISORDERS

The Eighth Annual Interdisciplinary Seminar on Diagnostic and Rehabilitative Aspects of Dizziness and Balance Disorders will be held December 4-7, 1996, in Denver, Colorado. For further information, contact Jane Wells, MA, 1996 Balance Meeting, 300 E Hampden Ave, Suite 401, Englewood, CO 80110; telephone (303) 788-4235; fax (303) 788-4234.

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FOURTH ANNUAL GOLF SEMINAR — ADVANCES IN OTOLARYNGOLOGY–HEAD AND NECK SURGERY

The Fourth Annual Golf Seminar — Advances in Otolaryngology–Head and Neck Surgery will be held March 11-16, 1997, at the Pinehurst Resort & Country Club in North Carolina. For information, contact Leslie Sullivan, Coordinator, University of Washington School of Medicine Virginia Merrill Bloedel Hearing Research Center, Box 357923, Seattle, WA 98195-7923; E-mail sullivan@u.washington.edu; telephone (206) 685-2962; fax (206) 616-1828.

Ann Otol Rhinol Laryngol 105:1996

COMPUTER-AIDED THREE-DIMENSIONAL RECONSTRUCTION IN HUMAN COCHLEAR MAPS: MEASUREMENT OF THE LENGTHS OF ORGAN OF CORTI, OUTER WALL, INNER WALL, AND ROSENTHAL'S CANAL

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This paper describes the application of computer-aided three-dimensional reconstruction to measurements of the length of the organ of Corti (mean ± SD, 35.58 ± 1.41 mm), scala tympani outer wall (40.81 ± 1.97 mm), scala tympani inner wall (18.29 ± 1.47 mm), and Rosenthal's canal center (15.98 ± 1.33 mm) in eight adult male cochleas. The Rosenthal's canal center ranged between 1¾ and 2 turns, did not appear to be linearly related to the organ of Corti, and lay near the basal end of the latter. The length of the organ of Corti measured with three-dimensional reconstruction differed by 7.6% ± 3.2% ("cutting angle difference") from that derived from traditional two-dimensional reconstruction on the plane perpendicular to the plane of section, and by 2.6% ± 1.7% ("geometric difference") from that derived from two-dimensional reconstruction on the axial plane at right angles to the modiolar axis.

KEY WORDS — anatomic measurement, cochlear length, computer model, cutting angle difference, geometric difference.

INTRODUCTION

Computer-aided three-dimensional (3-D) reconstruction can provide much valuable information about 3-D structures that are so small, fragile, and obscure that we cannot observe them without histologic processing and microscopic study. Three-dimensional computer models have been generated from histopathologic specimens, computed tomography (CT) scans, and magnetic resonance imaging. They make it easy to recognize 3-D shapes of structures and relationships among structures and enable us to quantify the distances (lengths), angles, areas, surface areas, volumes, etc. Recent publications have highlighted the potential for 3-D study of the structures within the temporal bone, mainly from histologic sections, to define important anatomic relationships aimed at surgical anatomy of the stapes,[1] facial nerve,[2] vestibular end organ,[3] and cochlea,[4,5] etc. We are utilizing 3-D reconstruction from CT scans of the temporal bone in cochlear implant candidates as part of the preoperative examination and surgical planning.[6,7] On the other hand, a majority of investigators have used the approximate two-dimensional (2-D) graphic reconstruction technique introduced by Guild[8] and Schuknecht[9] to map the organ of Corti (OC) for assessing cochlear function and pathology. However, we believe it is difficult to recognize 3-D structures properly by using 2-D graphic reconstruction. Takagi and Sando[10] described one problem of studying temporal bones using traditional 2-D methods: the "cutting angle" (the angle between the plane of section and the modiolar axis). They compared the length of the cochlea by 2-D and 3-D methods in a single case.

We generated 3-D models of temporal bone section structures on a personal computer. This system enables us to measure several parameters (distances, angles, areas, surface areas, cross-sectional areas, volumes, etc) on 3-D structures, regardless of the angle at which the specimen was cut. This paper reports our method for the 3-D reconstruction of the cochlea and the quantification of cochlear structures: OC, outer wall (OW), inner wall (IW), and Rosenthal's canal center (RCC) from the basal end to the apical end. Knowledge of these distances is important for the placement of cochlear implants, as implant depth is apparently a factor in subsequent performance.[11] The lengths of the OC measured by 3-D reconstruction were compared with those measured by two 2-D methods. The lengths and fractional lengths of each parameter could be calculated for each quarter turn and for each corresponding position of the OC. That is, each structure could be mapped onto the OC.

MATERIALS AND METHODS

Materials. Eight temporal bones were used in this

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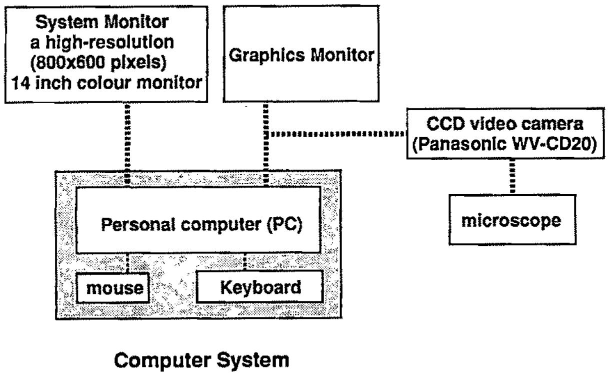

Fig 1. Block diagram of our system. Equipment consists of personal computer, high-resolution (800 × 600) 14-in color monitor as system monitor, graphics monitor, CCD video camera (Panasonic WV-CD20), and microscope.

study. They were obtained at postmortem examination and were from men ranging from 67 to 79 years of age. A brief medical history was obtained, and temporal bones from patients thought to have suffered otosclerosis, obliterative labyrinthitis, or congenital malformation, tumors, or fractures of the region were not included in this study. The bones were placed in formol-saline at 4°C for at least 48 hours, and then rinsed in distilled water. The cochleas were decalcified in 14% ethylenediaminetetraacetic acid in neutral buffered formalin and embedded in Spurr's resin. The blocked cochleas were sectioned at a thickness of 3 µm, and sections from every 130 µm were collected and stained with hematoxylin and eosin.

Preparation of 3-D Reconstruction Images. The equipment consisted of a personal computer, a high-resolution (800 × 600 pixels) 14-inch color monitor as system monitor, a graphics monitor, a charge-coupled device video camera (the Panasonic WV-CD20), and a microscope (Fig 1).

Temporal bone sections were viewed through the microscope and digitized through the video camera connected to the personal computer via a Data Translation DT2851 frame-grabber card, as previously reported.[6] The contrast and brightness of each image could be adjusted to optimize the contrast for the features of interest. With the cursor we manually marked and stored coordinates of the union of the pillar cells' heads as the OC, the most lateral point of the scala tympani as the OW, the most medial point of the scala tympani as the IW, and a point in the center of Rosenthal's canal as the RCC (Fig 2). Each set of data points describing the location of a feature in a section constituted an entry in the data file, with a corresponding line in an index file. Each index entry included the section number and feature number of the data set, plus the number of points in the data. After all relevant points of a given section had been stored, they could be displayed as a red overlay on the real-time image of the next serial section. Thus, the next section could be maneuvered until the best visual fit with all the borders of the previous section had been obtained. This position was then used for the point detection on the new section. Takagi and Sando[10] put reference holes adjacent to the temporal bone specimen before sectioning, but our system allows us to use general histologic series with no artificial reference points. The 3-D reconstruction was made after all features obtained from sequential sections were sorted. Displaying the stored points for the OC alone showed a pseudo–3-D spiral (Fig 3[8-10]).

Measurement of Length of OC. A distance map of the OC was made by summing the distances between successive pairs of points, starting at the basal end in the hook region (as 0) and continuing to the apical end. We excluded the few points that were clearly out of position. Furthermore, we measured the length of every quarter turn as defined by the following method.

Definition of Quarter Turns of OC. The schema for deciding the position of quarter turns of the OC is shown in Fig 4.[12] First of all, the furthest point from the basal end (A0) on the OC is defined as one half turn (A0.5), and a line L0/0.5 is drawn through A0 and A0.5. The furthest point from L0/0.5 in the lower basal turn is one quarter turn (A0.25); the furthest point in the upper basal turn is three quarter turns (A0.75, Fig 4A). If the perpendicular line from L0/0.5 to A0.75 is extended and called L0.75, then the furthest point in the next half turn beyond A0.75 is A1.0, the end of the basal turn, and the perpendicular line from L0.75 to it is L1.0. Similarly, we now proceed iteratively, extending the current line, finding the point furthest from it in the next half turn, labeling that point as the end of the next quarter turn and the line perpendicular to it as the next current line (eg, Fig 4B).

In traditional 2-D reconstruction methods, Bredberg's[12] definition has been used (Fig 4C). He represented the locations based on the angle around the helicotrema. But significant error could be expected from this subjective choice of a center, because the OC is not concentric around it. The eccentricity caused by using the helicotrema–hook region line as an axis affects the axis crossings, and thus, the quarter turn points up to the apical end. On the other hand, our method does not need to set the axis of revolution. It works with only the points in the OC coordinate set, and it is easy to determine a quarter turn from a half turn as described above.

Measurement of Lengths of Every Quarter Turn and Every 4% Interval of OC. For each OC quarter turn, the lengths of the OC, OW, IW, and RCC were

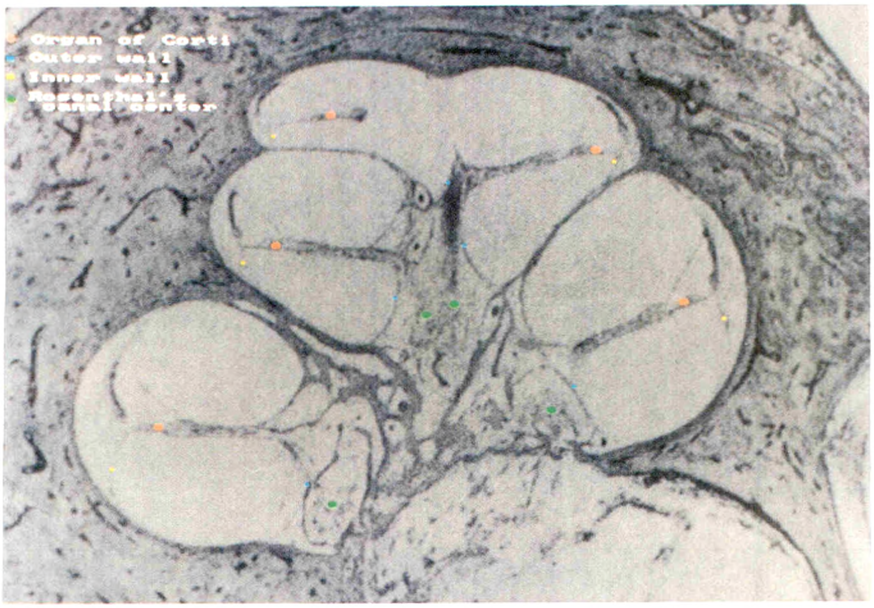

Fig 2. Digitization of cochlear structures. Temporal bone sections were viewed through microscope and digitized through video camera connected to personal computer via Data Translation DT2851 frame-grabber card. Organ of Corti (OC, pink dots) was defined as union of pillar cells' heads and was marked manually with cursor on display. Outer wall (OW, yellow points), inner wall (IW, light blue points), and Rosenthal's canal center (RCC, light green dots) were also marked manually.

measured and presented as absolute values and as fractions of the totals. The corresponding positions of the OW, IW, and RCC were selected on the plane perpendicular to the line of the OC at each quarter turn point (Fig 5). Furthermore, in view of individual differences, the lengths of the OW, IW, and RCC corresponding to every 4% interval of the OC were measured the same as quarter turn points.

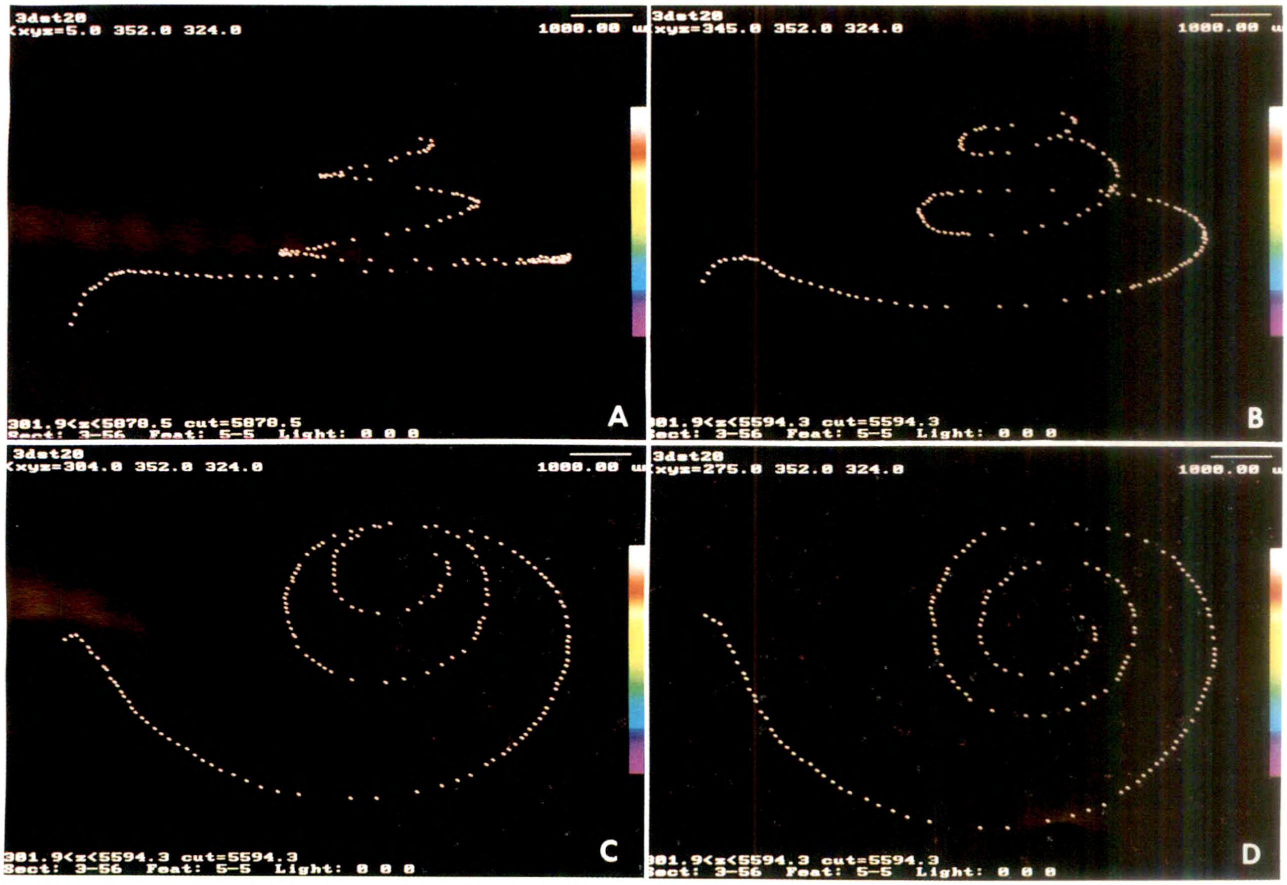

Fig 3. Comparison of 3-D and 2-D reconstruction (OC, No. 20). Displaying stored points for OC alone, 3-D reconstruction showed 3-D spiral. A) Side aspect (modiolar axis vertical). B) Oblique aspect, modiolar axis tilted 20° toward observer. C) Oblique aspect along original plane of section; "cutting angle" is 29° in this case; this is view as used by Guild[8] and Schuknecht.[9] D) Axial view aspect, projected onto plane perpendicular to modiolar axis ("cutting angle" compensated to 0° as described by Takagi and Sando[10]).

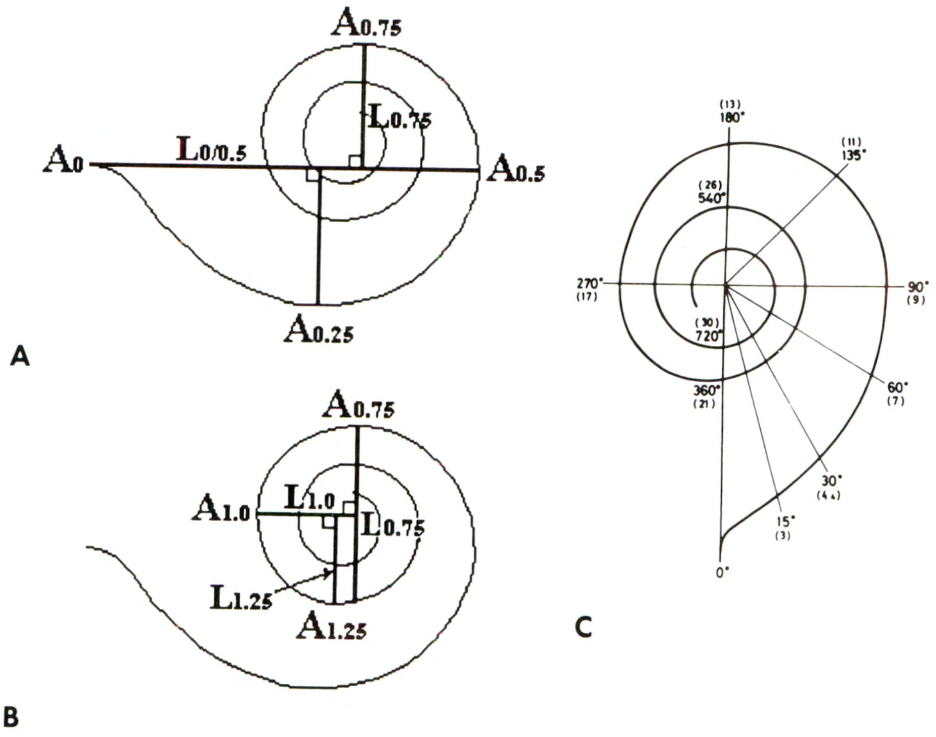

Fig 4. Schema for defining position of every quarter turn of OC. Details are described in text. A) Points in basal turn. B) Points in upper basal and middle turns. A0 — basal end of basal turn; A0.25 — point of one quarter turn; A0.5 — point of one half turn; A0.75 — point of three quarter turns; A1.0 — point of one turn; A1.25 — point of $1^1/4$ turns; L0.25 — line at one quarter turn; L0.5 — line at one half turn; L0.75 — line at three quarter turns; L1.0 — line at one turn; L1.25 — line at $1^1/4$ turns. C) 2-D reconstruction described by Bredberg.[12] It shows locations of different angular measurements along OC. He set helicotrema as axis of revolution of OC, but OC is eccentric around helicotrema, and his quarter turn points are thus closer to apical end than ours.

Measurement of Lengths of OC by 2-D Reconstruction Methods. For comparison, we also measured lengths of the OC from two 2-D reconstruction methods. In one case, it was performed by traditional 2-D reconstruction on the plane perpendicular to the plane of section[8,9] (Fig 3C), and in another case, 2-D reconstruction at right angles to the modiolar axis by rotation of the OC in 3-D space[10] (Fig 3D). In the first case, we call the angle between the modiolar axis and the plane of section the "cutting angle," and the difference attributed mainly to this the "cutting angle difference"; it represents the difference between 2-D reconstruction on the plane perpendicular to the plane of section and 3-D reconstruction. In the second case, we call the difference attributed to the pseudospiral structure of the OC the "geometric difference."

RESULTS

Lengths of OC, OW, IW, and RCC. Table 1 shows the numbers of turns of the OC and RCC, and the total lengths of the OC, OW, IW, and RCC. The number of turns of the OC ranged from $2^5/8$ (in six cochleas) to 3, with an average of 2.69. The number of turns on the

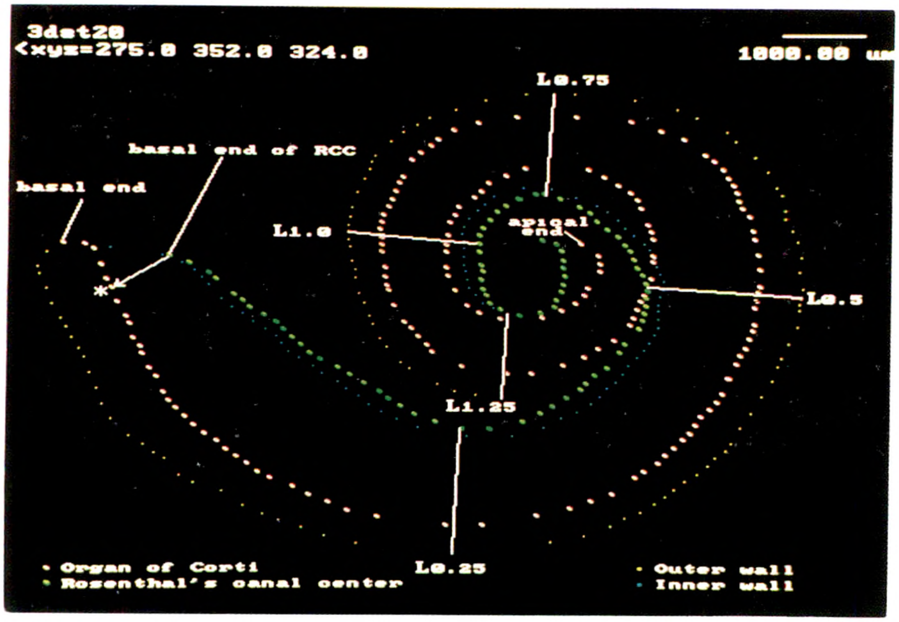

Fig 5. Graphic reconstruction and definition of every quarter turn of OC, OW, IW, and RCC. Five lines show positions of quarter turns from basal end to $1^1/4$ turns on plane at right angles to modiolar axis. IW and RCC are not drawn beyond $1^1/4$ turns. Pink dots — OC; yellow points — OW; light blue points — IW; light green dots — RCC; asterisk — position on OC corresponding to basal end of RCC.

TABLE 1. NUMBERS OF TURNS ON OC AND RCC, AND TOTAL LENGTHS OF OC, OW, IW, AND RCC FROM BASAL END

Cochlea No.	*Turns of OC*	*Turns of RCC*	*Length of OC (mm)*	*Length of OW (mm)*	*Length of IW (mm)*	*Length of RCC (mm)*
4	$2^{5}/8$	$1^{6}/8$	34.15	37.93	17.51	15.38
6	$2^{5}/8$	$1^{6}/8$	34.77	40.60	17.53	15.47
7	$2^{6}/8$	$1^{7}/8$	34.52	39.67	17.04	14.73
8	$2^{5}/8$	2	35.44	40.20	18.27	16.96
9	$2^{5}/8$	2	34.16	39.52	16.99	14.73
13	$2^{5}/8$	$1^{7}/8$	36.75	41.51	18.04	15.12
17	3	2	36.98	43.22	21.17	18.38
20	$2^{5}/8$	$1^{6}/8$	37.90	43.81	19.81	17.07
Mean	$2^{5.5}/8$	$1^{7}/8$	35.58	40.81	18.29	15.98
	(2.69)	(1.88)				
SD	0.11	0.12	1.41	1.97	1.47	1.33
Max	3	2	37.90	43.81	21.17	18.38
Min	$2^{5}/8$	$1^{6}/8$	34.15	37.93	16.99	14.73

OC — organ of Corti, RCC — Rosenthal's canal center, OW — outer wall, IW — inner wall.

RCC were from $1^{3}/4$ to 2, with an average of 1.88. The total lengths of the OC ranged between 34.15 and 37.90 mm, with an average of 35.58 ± 1.41 mm (mean ± SD). Similarly, the total lengths of the OW, IW, and RCC were 40.81 ± 1.97, 18.29 ± 1.47, and 15.98 ± 1.33 mm, respectively.

The lengths and the percentages of totals of the OC, OW, IW, and RCC from the basal end to every quarter turn are shown in Table 2 and Fig 6. The average lengths of the basal turn, the second turn, and the apical turn of the OC were 20.91 mm (58.8%), 10.15 mm (28.5%), and 4.52 mm (12.7%), respectively. Rosenthal's canal is not found at the basal end of the OC (within 1.90 ± 0.38 mm from the basal end), or beyond the second turns. The RCC ranged between $1^{3}/4$ and 2 turns; the average length of the basal turn was 12.87 mm (80.6%); that of the second turn was 3.11 mm (19.4%). The halfway point of the OC is located at about three quarters of its basal turn, and

TABLE 2. LENGTHS, AND PERCENTAGE LENGTHS IN PARENTHESES, OF OC, OW, IW, AND RCC FROM BASAL END, CALCULATED AT EVERY QUARTER TURN

Turns	*OC*	*OW*	*IW*	*RCC*
0	0	0	0	(0)
0.25	7.75 ± 0.62	7.92 ± 0.83	6.03 ± 0.40	5.81 ± 0.47
	(21.8 ± 1.4)	(19.4 ± 1.7)	(33.1 ± 2.7)	(36.5 ± 3.28)
0.5	12.94 ± 0.87	13.85 ± 1.13	9.05 ± 0.82	8.92 ± 0.68
	(36.3 ± 1.9)	(33.9 ± 2.2)	(49.6 ± 3.63)	(55.9 ± 3.3)
0.75	17.48 ± 0.96	19.25 ± 1.31	11.67 ± 0.95	11.29 ± 0.92
	(49.1 ± 1.4)	(47.2 ± 1.9)	(63.8 ± 2.1)	(70.7 ± 2.0)
1	20.91 ± 1.08	23.32 ± 1.52	13.53 ± 1.16	12.87 ± 1.07
	(58.8 ± 1.3)	(57.1 ± 2.1)	(74.0 ± 2.6)	(80.6 ± 2.1)
1.25	23.97 ± 1.23	27.07 ± 1.64	15.05 ± 1.20	14.09 ± 1.17
	(67.3 ± 1.4)	(66.3 ± 1.9)	(82.3 ± 2.61)	(88.2 ± 1.7)
1.5	26.52 ± 1.30	30.21 ± 1.82	16.14 ± 1.24	15.02 ± 1.19
	(74.5 ± 1.3)	(74.0 ± 2.0)	(88.2 ± 2.2)	(97.4 ± 1.1)
1.75	28.85 ± 1.36	33.18 ± 1.86	16.99 ± 1.21	15.58 ± 1.28
	(81.1 ± 1.2)	(81.3 ± 2.2)	(93.0 ± 2.3)	(97.4 ± 1.1)
1.875				15.98 ± 1.33
				(100)
2	31.06 ± 1.45	35.81 ± 2.01	17.67 ± 1.27	
	(87.3 ± 1.6)	(87.8 ± 2.7)	(96.7 ± 1.3)	
2.25	32.95 ± 1.52	38.37 ± 2.05	17.96 ± 1.27	
	(92.6 ± 1.5)	(94.0 ± 2.4)	(98.2 ± 1.1)	
2.5	34.63 ± 1.55	40.25 ± 1.88	18.17 ± 1.40	
	(97.3 ± 1.2)	(98.6 ± 0.9)	(99.4 ± 0.5)	
2.625	35.58 ± 1.41	40.81 ± 1.97	18.29 ± 1.47	
(Total)	(100)	(100)	(100)	

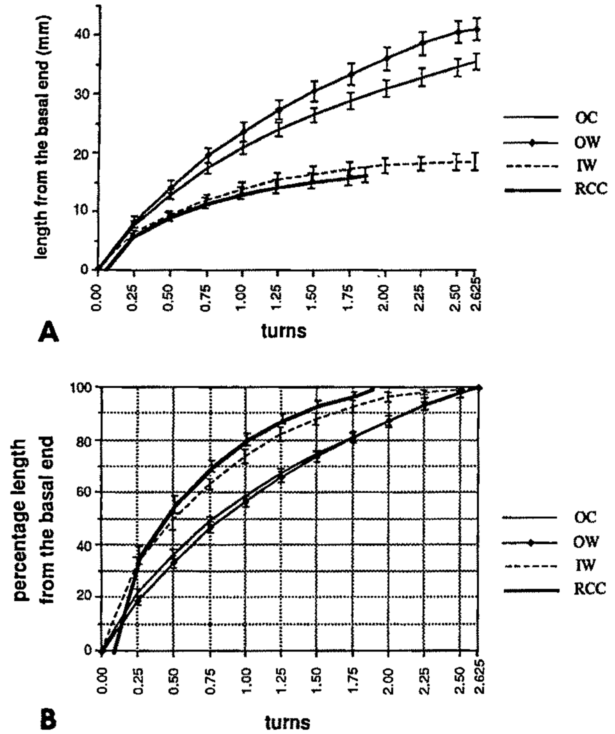

Fig 6. A) Lengths and B) percentage lengths of OC, OW, IW, and RCC from basal end, calculated at every quarter turn.

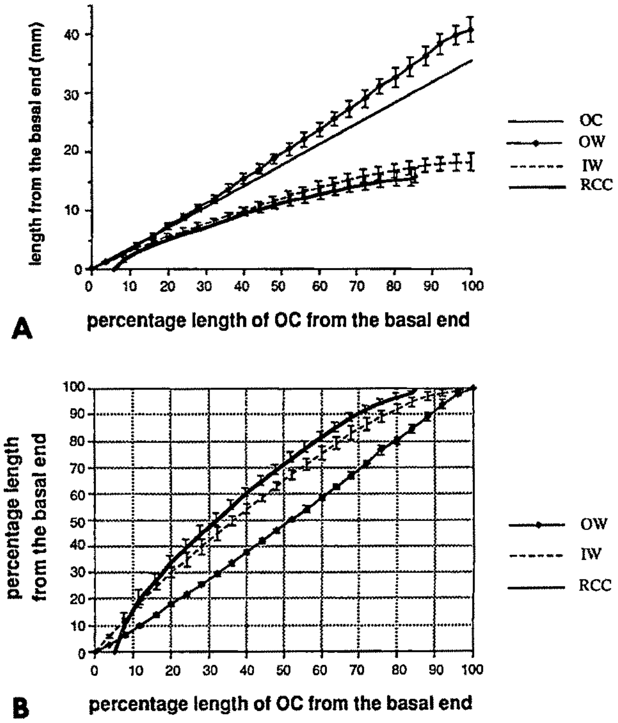

Fig 7. A) Lengths and B) percentage lengths of OW, IW, and RCC at 4% intervals along OC. RCC does not appear to be linearly related to OC, and shifts toward basal end.

that of the RCC is just before half of the OC basal turn.

The lengths and the percentages of the totals of the OW, IW, and RCC corresponding to the OC positions at 4% intervals are shown in Fig 7. The RCC started from the point corresponding to 5.3% ± 1.9% of the OC; the points at 30%, 50%, and 70% of the RCC corresponded to those at 17.4%, 21.9%, and 48.6% of the OC, respectively; and the apical end of the RCC was at 85.0% of the OC length. The RCC does not appear to be linearly related to OC, lying closer to the basal end of the latter. Comparing the percentage lengths of the OC, OW, IW, and RCC, that of the OW is close to that of the OC, and that of the IW is close to that of the RCC generally, as would be expected from their physical proximity. The standard deviations of lengths at each quarter turn point and at 4% intervals tend to increase closer to the apical end, because the lengths of the eight cochleas are somewhat different.

Lengths of OC by 2-D Reconstruction Methods. Table 3 lists the lengths of the OC measured by 2-D reconstruction on the plane perpendicular to the plane of section (A*, Fig 3C), by 2-D reconstruction at right angles to the modiolar axis by rotation of the OC in 3-D space (B*, right-angle plane, Fig 3D), and by 3-D reconstruction (C). The value of A* was 32.88 ± 1.29

TABLE 3. COMPARISON OF LENGTHS OF OC BY 2-D AND 3-D METHODS

Cochlea No.	*4*	*6*	*7*	*8*	*9*	*13*	*17*	*20*	*Mean*	*SD*
2-D length (mm) on plane perpendicular to plane of section (A*)	31.58	31.02	33.17	33.96	31.94	33.43	34.88	33.06	32.88	1.29
"Cutting angles" (degree)†	13.5	9.2	5.8	10.2	5.4	7.8	7	29	10.99	7.73
2-D length (mm) at axial plane (B*)	33.55	33.74	33.69	34.74	33.47	34.32	36.40	37.47	34.67	1.49
3-D length (mm) (C)	34.15	34.77	34.52	35.44	34.16	36.75	36.98	37.90	35.58	1.44
Percentage "cutting angle difference"† (C–A*)/C	7.53	10.79	3.90	4.17	6.48	9.04	5.69	12.78	7.55	3.15
Percentage "geometric difference"† (C–B*)/C	1.76	2.96	2.40	1.96	2.03	6.63	1.57	1.13	2.56	1.73

2-D reconstructions were performed by two methods: A* was traditional 2-D reconstruction on plane perpendicular to plane of section, and B* was 2-D reconstruction at right angles to modiolar axis by spatial rotation of 3-D model. 3-D results are denoted by C.

†Details are described in text.

TABLE 4. SUMMARY OF STUDIES OF OC LENGTH IN HUMANS

Authors	Date	Length of OC (mm)			No. of Cases	Reference Specimens	Method of Measurement
		Mean	SD	Range			
Hardy[13]	1938	31.5	2.3	25.3 - 35.5	68	Human	2-D reconstruction method
Walby[14]	1985	32.6	2.1	30.1 - 36.4	20	Human	2-D reconstruction method
Pollak et al[15]	1987	28.4	3.4	24.0 - 33.5	9	Human	2-D reconstruction method
Retzius[16]	1884	33.5	0.8	32.0 - 34.0	5	Human	Direct method
Bredberg[12]	1968	33.2	3.8	28.2 - 40.1	9	Fetal	Direct method
		34.7	1.1	33.5 - 37.6	18	Adult	
Wright et al[17]	1987	32.8	2.6	28.8 - 36.6	12	Human	Direct method
Takagi and Sando[10]	1989	36.4			1	Man	3-D reconstruction method
Sato et al[18]*	1991	37.1	1.6	33.5 - 38.9	9	Men	3-D reconstruction method
		32.3	1.8	29.7 - 34.7	9	Women	
This study		35.6	1.4	34.2 - 37.9	8	Men	3-D reconstruction method

*Sato et al[18] measured length of outer and inner margins of basilar membrane and used their average.

mm; the "cutting angle difference" was 7.6% ± 3.2%, and the "cutting angle" was 11.0° ± 7.7°. The value of B* was 34.67 ± 1.49 mm, and the "geometric difference" was 2.6% ± 1.7%. The lengths by both 2-D methods (A* and B*) were shorter than that from the 3-D method (C). Among the eight cochleas, the OC of No. 20, which had the highest "cutting angle" (29°), had the highest "cutting angle difference" (12.8%, A*: 33.06 mm, Fig 3C, C: 37.90 mm); the "geometric difference" was 1.13% (B*: 37.47 mm, Fig 3D).

DISCUSSION

There are several reports about computer-aided reconstruction of the temporal bone and on the length of the human cochlea. Three types of methods of measuring the length have been described. First is the 2-D reconstruction method (indirect method) used by Guild,[8] Schuknecht,[9] Hardy,[13] Walby,[14] and Pollak et al[15]; second is the direct method (surface specimen technique) used by Retzius,[16] Bredberg,[12] and Wright et al[17]; and last is 3-D reconstruction, used by Takagi and Sando[10] and Sato et al[18] and in this study. The measured lengths of the human OC from these studies are listed in Table 4.[10,12-18]

The first method, the traditional 2-D reconstruction method, was developed by Guild[8] and revised by Schuknecht[9]; relevant features marked on all serial sections are reconstructed and analyzed in one plane. Guild[8] described the prerequisites for use of the 2-D reconstruction method as 1) good fixation and embedding of the cochlea and 2) serial sections of the cochlea in the approximate plane of the modiolar axis. He estimated the shortening error in graphic reconstruction of the guinea pig as less than 5%. This shortening error, which we call "chord error," arises because the real distance between two points on the OC is that along the arc of the spiral connecting the points; the measured distance is that along the chord of the arc connecting the points. Thus, the lengths measured by the 2-D reconstruction method tend to be shorter than the true lengths. Other factors may also account for differences in measured length. Takagi and Sando[10] suggested the cutting angle as one source of error, because most horizontal temporal bone sections are cut parallel to the middle cranial fossa surface, which is not the horizontal plane anatomically. Also, the modiolus itself is too thick to permit accurate judgment from midmodiolar sections as to whether it was cut exactly along the axis. In one particular example of a cochlea specimen with a 35° cutting angle, they measured and compared the length of the OC by traditional 2-D versus 3-D reconstruction. The "cutting angle difference" was considerable (15.4%). In our cases also, the 3-D method yields considerably greater OC lengths; the average cutting angle difference was 7.6% (32.9 mm versus 35.6 mm). We investigated the correlation between the cutting angle and the cutting angle difference. In our eight cases there was not a not-quite-significant correlation ($r = .696, R^2 = .484, p = .0554$). But when we included Takagi and Sando's case as the ninth, a significant correlation was found ($r = .841, R^2 = .708, p = .0045$). Our cases included seven with a relatively small cutting angle (5.4° to 13.5°), so that no certain relation was noted, but the above results demonstrate that Takagi and Sando's[10] one case strongly influences the analysis. Viewing a cochlea with the same cutting angle, we can easily understand that the 2-D reconstruction varies considerably according to the plane of section, whether it is close to the major axis (the direction of $L_{0/0.5}$ in Fig 4) or to the minor axis (the direction of $L_{0.25}$ and $L_{0.75}$ in Fig 4), and this results in various cutting angle differences. But higher cutting angles lead unequivocally to higher cutting angle differences, as in Takagi and Sando's case and our case 20. In conclusion, the length of the cochlea on 2-D reconstruction is much shorter than the actual value when the plane of section is oblique to the midmodiolar axis, and the major source of error in 2-D reconstructions may be the cutting angle.

When the cutting angle was compensated to be 0° by rotation of the 3-D model in space, the length of the OC was 34.7 ± 1.5 mm; the average difference between this and the 3-D results was 2.6%. This difference is the "geometric difference" due to the pseudospiral structure of the cochlea; in brief, the cochlear spiral changes its radius of curvature, axis of revolution, and inclination from the basal end to the apical end, as previously described by Schuknecht.[9] He made a geometric side-view projection for the upper 2½ turns of the cochlea of a cat — excluding the hook region — and estimated a difference of 2.8% between the length in the simple top-view reconstruction and the length of this projection. Takagi and Sando[10] reported the 2-D length of a cochlea made from a projection of a 3-D reconstruction method was very close to the 3-D value (the geometric difference was 0.3%). Similarly, in our data there is a smaller geometric difference. But the data from 2-D reconstruction include some geometric difference whenever the cutting angle requires compensation to 0°, and results in shorter-than-actual values.

The second method, for direct measurement of cochlear length, was described first by Retzius,[16] who used a micrometer, and later Bredberg[12] and Wright et al,[17] who measured the cochlea using surface specimens (measurement by looking straight at the OC under a microscope). This technique seems to be more reliable than indirect measurement (traditional 2-D reconstruction); the average OC length varies from 32.8 mm to 34.7 mm and is longer than that from indirect measurement. But compared with our data, it is still shorter, because there is still the possibility of difference due to the viewing angle — similar to the cutting angle difference — or to destruction or loss of parts of the OC during the segmentation of the cochlea.

Bredberg[12] measured the length of the OC from the basal end according to the angle of the turn around the helicotrema (Fig 4C). The percentage lengths in the basal turn (61.5%) and basal and middle turns (88.5%) were slightly longer than ours (58.8% and 87.3%, respectively). The OC is not concentric around the helicotrema, which Bredberg set as the center of the angle to be measured, and his quarter turn points are always further toward the apical end than ours.

The third method, 3-D reconstruction, can easily be done by computers. The length of 3-D structures can be measured by procedures described by Takagi and Sando,[10] Sato et al,[18] and this paper. Sato et al[18] measured the lengths of the outer and inner margins of the basilar membrane and calculated the average, and reported that the mean cochlear length was significantly longer in males (37.1 ± 1.6 mm) than in females (32.3 ± 1.8 mm). Their measured parameters are not directly comparable with ours, but their results were longer than the previous ones from other methods. Although our method requires considerable time and practice to make a 3-D reconstruction, the length between any two points can be calculated quickly by selecting the points. Furthermore, the calculated lengths have neither a "cutting angle difference" nor a "geometric difference." However, the 3-D method still retains the possibility of "chord error," which decreases as the distance between successive sections becomes shorter.

Finally, a possible source of error that affects most methods could be shrinkage during the embedding process. The fact that the lengths measured by 3-D methods are generally greater than those from other methods shows at least that the histologic processing for the former does not cause more shrinkage artifact than that for the latter. More specifically, we decalcify at room temperature with ethylenediaminetetraacetic acid in formalin; this method is slow, but causes a minimum of artifact. Even after decalcification, the tissue of the otic capsule is more rigid than "normal" soft tissue, and embedding usually is done as soon as the decalcification is sufficient for sectioning. Although we feel that there is a minimum of shrinkage involved in the process, clearly, more systematic studies need to be done.

Despite differences in absolute cochlear length, the percentage distances along the basilar membrane have been reported to be similar in cat[19] and chinchilla.[20] If this hypothesis is true, the frequency map on the basilar membrane may be different in cochleas of different lengths. Generally, cochlear length refers to the length of the OC, so in this study the lengths of the OW, IW, and RCC were mapped onto the OC at 4% intervals. Our computer-aided 3-D reconstructions revealed that Rosenthal's canal is much shorter than the OC; the lengths of the RCC and IW were only half of the OC's length. The RCC started at the position of 5.3% of the length of the OC and ended within two turns — at 85.0% of the OC — as described by Ariyasu et al.[21] Ariyasu et al also reported that the spiral ganglion dendrites within the osseous spiral lamina project radially in the basal turn and become increasingly oblique to the axis of the modiolus as the ganglion extends apically. Our results corroborate theirs. According to our comparative analysis on the lengths of the OC and RCC (Fig 7), the percentage length of the RCC was not linearly related to that of the OC; the RCC tended to shift toward the basal end. There are few reports in the literature on the length of the human Rosenthal's canal. The 12 mm reported by Pfingst[22] was shorter than our finding of 16.0 ± 1.3 mm, probably, again, because of differences between 2-D and 3-D reconstructions, as previously described.

These results should clearly influence the design of future cochlear implants. Insertion along the inner scala tympani wall is clearly preferable because of its closer proximity to Rosenthal's canal and the considerably shorter insertion depth necessary to achieve stimulation of the apical end of Rosenthal's canal.

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REFERENCES

1. Takahashi H, Sando I. Three-dimensional surgical anatomy for stapes surgery computer-aided reconstruction and measurement. Laryngoscope 1992;102:1159-64.
2. Yasumura S, Takahashi H, Sando I, Aoki H, Hirsch BE. Facial nerve near the external auditory meatus in man: computer reconstruction study — preliminary report. Laryngoscope 1993; 103:1043-7.
3. Takagi A, Sando I. Computer-aided three dimensional reconstruction and measurement of the vestibular end-organs. Otolaryngol Head Neck Surg 1988;98:195-202.
4. Takahashi H, Sando I. Computer-aided 3-D temporal bone anatomy for cochlear implant surgery. Laryngoscope 1990; 100:417-21.
5. Takahashi H, Sando I, Takagi A. Computer-aided three dimensional reconstruction and measurement for multiple-electrode cochlear implant. Laryngoscope 1990;100:1319-22.
6. Seldon HL. Three-dimensional reconstruction of temporal bone from computed tomographic scans on a personal computer. Arch Otolaryngol Head Neck Surg 1991;117:1158-61.
7. Dahm MC, Seldon HL, Pyman BC, Clark GM. 3-D reconstruction of the temporal bone in cochlear implant surgery. In: Yanagihara N, Suzuki J, eds. Transplants and implants in otology. Amsterdam, the Netherlands: Kugler Publications, 1992: 271-5.
8. Guild SR. A graphic reconstruction method for the study of the organ of Corti. Anat Rec 1921;22:141-57.
9. Schuknecht HF. Technique for study of cochlear function and pathology in experimental animals. Arch Otolaryngol 1953; 58:377-97.
10. Takagi A, Sando I. Computer-aided three dimensional reconstruction: a method of measuring temporal bone structures including length of the cochlea. Ann Otol Rhinol Laryngol 1989; 98:515-22.
11. Blamey PJ, Pyman BC, Gordon M, et al. Factors predicting postoperative sentence scores in postlinguistically deaf adult cochlear implant patients. Ann Otol Rhinol Laryngol 1992;101: 342-8.
12. Bredberg G. Cellular pattern and nerve supply of human organ of Corti. Acta Otolaryngol [Suppl] (Stockh) 1968(suppl 236):34-9.
13. Hardy M. The length of the organ of Corti in man. Am J Anat 1938;62:291-311.
14. Walby AP. Scala tympani measurement. Ann Otol Rhinol Laryngol 1985;94:393-7.
15. Pollak A, Felix H, Schrott A. Methodological aspects of quantitative study of spiral ganglion cells. Acta Otolaryngol [Suppl] (Stockh) 1987(suppl 436):37-42.
16. Retzius G. Das Gehörorgan der Wirbeltiere. 2. Das Gehörorgan der Reptilien, der Vögel und der Söugetiere. Stockholm, Sweden: Samson & Wallin, 1884.
17. Wright A, Davis A, Bredberg G, Ulehlova L, Spencer H. Hair cell distributions in the normal human cochlea. Acta Otolaryngol [Suppl] (Stockh) 1987(suppl 436):15-24.
18. Sato H, Sando I, Takahashi H. Sexual dimorphism and development of the human cochlea. Computer 3-D measurement. Acta Otolaryngol (Stockh) 1991;111:1037-40.
19. Liberman CC. The cochlear frequency map for the cat: labeling auditory-neural fibers of known characteristic frequency. J Acoust Soc Am 1982;75:1441-9.
20. Bohne BA, Carr CD. Location of structurally similar areas in chinchilla cochleas of different lengths. J Acoust Soc Am 1979;66:411-4.
21. Ariyasu L, Galey FR, Hilsinger R, Byl FM. Computer-generated three-dimensional reconstruction of the cochlea. Otolaryngol Head Neck Surg 1989;100:87-91.
22. Pfingst BE. Stimulation and encoding strategies for cochlear prostheses. Otolaryngol Clin North Am 1986;19:219-36.

Ann Otol Rhinol Laryngol 105:1996

PLASMA PLATELET-DERIVED GROWTH FACTOR: PRELIMINARY STUDY OF A POTENTIAL MARKER IN HEAD AND NECK CANCER

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The role of growth factors in the development and spread of head and neck cancers has received little attention. Platelet-derived growth factor (PDGF) is a potent mitogen that is released normally in wound healing, but is also secreted by human malignant epithelial cells. In breast and ovarian carcinomas, elevated plasma PDGF has correlated with a poorer prognosis. This preliminary study was designed 1) to determine if PDGF is elevated in the plasma of patients with squamous cell carcinoma of the head and neck and 2) to determine whether there is any change in levels following surgical ablation. The PDGF was measured by radioimmunoassay in 18 patients with head and neck squamous cell carcinoma and in 12 normal controls. In the control group the mean level was 7.4 fmol/100 μL (range 2.8 to 12.5, median 7.6), in spite of the fact that PDGF is reportedly not measurable in normal subjects. The mean PDGF level in the cancer patients was 20.4 fmol/100 μL (range 4.1 to 35.7, median 20.5) and as compared to the control group was significantly elevated (median two-sample test, $p < .001$). Of the 20 cancer patients, only 4 had levels less than 12.5 fmol/100 μL. Moreover, 2 of these had undergone prior radiotherapy. In all cases, PDGF levels decreased significantly after surgery. These results support the importance of the further investigation of plasma PDGF levels as a potential biomarker to evaluate efficacy of treatment, possibly to aid in the detection of tumor recurrence, and even to be a potential indicator of tumor aggressiveness.

KEY WORDS — biomarker, growth factor, head and neck carcinoma, platelet-derived growth factor, radioimmunoassay.

The role of growth factors in the development and spread of head and neck cancers has received little attention. Polypeptide growth factors are small proteins produced by normal cells that regulate cell growth and differentiation. Platelet-derived growth factor (PDGF) is an extraordinary mitogen and chemotactic factor for mesenchymally derived connective tissue–forming cells and is secreted by platelets and other cells.[1] It is a heterodimer encoded by two different genes located on chromosomes 7 and 22.[2] Secretion of PDGF by malignant tissue can increase cell proliferation, inflammation, and tissue remodeling.

Messenger RNA of PDGF has been detected in 30 of 31 head and neck malignancies[3] and is also present in esophageal carcinoma.[4] In breast carcinoma patients, elevated plasma levels of PDGF measured by radioimmunoassay (RIA) correlated with increased metastases and decreased survival.[5] Furthermore, the greater the PDGF level, the greater the number of metastases. In one study, in all the patients with elevated plasma PDGF, immunohistochemical staining of tissue demonstrated PDGF.[6] Similarly, in ovarian carcinoma, PDGF expression by immunohistochemical staining is associated with a worse prognosis.[7]

One can measure PDGF in blood plasma by means of a commercially available RIA. The RIA is based on competition between the plasma PDGF and iodine 125 (^{125}I)–labeled PDGF for a limited number of binding sites on a specific antibody. A second antibody reaction is performed to permit the separation of the antibody-bound fraction by centrifugation. Measurement of the radioactivity in the resulting pellet permits calculation of the serum PDGF concentration. It is sensitive to 1.56 to 200 fmol per tube. In normal subjects the level is not detectable.[5] This study was designed to determine if PDGF is detectable in the plasma of patients with head and neck cancer and, if it is detectable, whether there is an alteration in the level after surgical ablation.

MATERIALS AND METHODS

Patients with squamous cell carcinoma of the mucosa of the upper aerodigestive tract treated at the University of Cincinnati Medical Center (UCMC) and Cincinnati Veterans Affairs Medical Center were offered enrollment in the study. Patients with a history of recent aspirin use or anticoagulant therapy were excluded. The study was performed in accordance with the UCMC institutional review board's protocol and all patients gave informed consent.

All patients' tumors were staged prior to surgery and 3 mL of blood was drawn into a tube containing 7.5 mmol/L ethylenediaminetetraacetic acid. The blood was centrifuged immediately and the plasma

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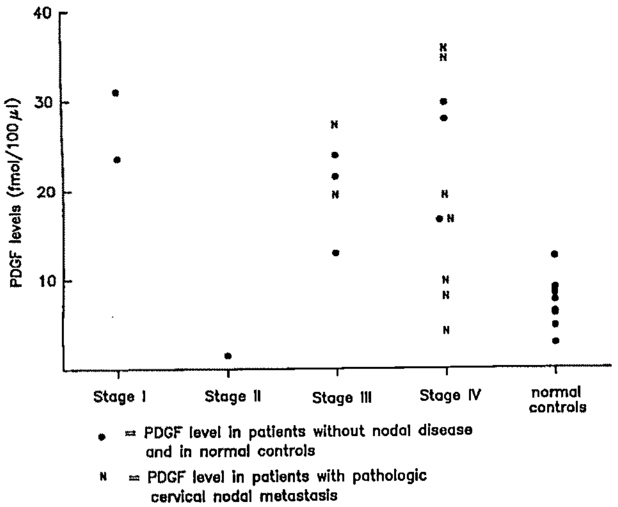

Plasma level of platelet-derived growth factor (PDGF) in cancer patients and normal controls.

stored at –20°C until the RIA was performed in the radiobiology laboratory at UCMC. The PDGF ^{125}I assay system (Amersham Corp, Arlington Heights, Ill) was utilized in accordance with the manufacturer's instructions. The assay is sensitive to 0.8 fmol. Cross-reactivity with related compounds is as follows: β-thromboglobulin <0.29%, transforming growth factor β <0.078%, platelet factor 4 <0.057%, and epithelial growth factor <0.0004%. Six to 10 weeks after surgery, additional samples were obtained for PDGF measurement.

We also measured PDGF in a control group of 12 healthy volunteers. This group consisted of 7 men and 5 women with a mean age of 35 years (range 19 to 57).

Eighteen patients were enrolled in the study prior to surgery. These patients had a mean age of 61 years (range 43 to 78) and there were 13 men and 5 women. The primary sites of the squamous cell carcinomas were oral cavity (7), oropharynx (4), supraglottis (2), glottis (2), nasopharynx (1), maxillary sinus (1), and unknown primary with nodal metastases (1). Nine patients had nodal disease and none had distant metastases. Two patients were stage I, 1 was stage II, 5 were stage III, and 10 were stage IV. Two patients had undergone prior radiotherapy for cure, and 1 patient had had a renal transplant and was on immunosuppressive therapy.

RESULTS

In the control group, PDGF was detected in all the plasma samples. The mean level was 7.4 fmol/100 µL, with a range from 2.8 to 12.5 and a median of 7.6. In the 18 patients with head and neck cancer, PDGF was detected in all the plasma samples except for the sample from the patient receiving immunosuppressive therapy. The mean level was 20.4 fmol/100 µL, with a range from 4.1 to 35.7 and a median of 20.5 (see Figure). The PDGF levels of the cancer patients and the control group were compared by a median two-sample test and achieved significance ($p<.001$).

PDGF LEVELS BEFORE AND AFTER SURGERY IN PATIENTS WITH INCREASED INITIAL LEVELS

		PDGF Level (fmol/100 µL)	
Stage	*Site*	*Before Surgery*	*After Surgery*
T1N0M0	Oral cavity	23.6	16.9
T3N0M0	Oropharynx	28.9	21.6
TxN3M0	Unknown	16.6	5.1
T3N1M0	Supraglottis	27.3	13.1
T4N2bM0	Oral cavity	34.6	17.1

PDGF — platelet-derived growth factor.

The highest PDGF level measured from the plasma in the control group was 12.5 fmol/100 µL; only 4 of the 18 cancer patients had PDGF levels below that. These 4 patients included the patient receiving immunosuppressants and the only 2 patients that had previously received radiotherapy. In addition, however, a patient with a T2N2bM0 squamous cell carcinoma of the oropharynx who received no prior therapy was noted to have a low PDGF level.

The PDGF level did not correlate with T stage or N stage. Although the highest levels were seen in patients with T4 disease, there were patients with T1 disease with PDGF levels above 30 fmol/100 µL. In the 9 patients that had cervical lymph node involvement confirmed by histopathology, PDGF levels ranged from 4.1 to 35.7 fmol/100 µL (see Figure).

The PDGF levels were measured after surgical therapy in 6 patients. This included the immunosuppressed patient, whose level before surgery was not measurable, but after surgery was measured at a low level of 7.9 fmol/100 µL. The other patients all had declines in their PDGF level (see Table).

DISCUSSION

A biomarker for head and neck cancer, such as carcinoembryonic antigen in colon cancer, can potentially be used to detect the initial cancer, to determine the treatment efficacy, and to monitor for disease recurrence. It could also be of value as a prognostic indicator of tumor aggressiveness. The previously documented presence of aberrant PDGF in the tumors of head and neck cancer patients, and the relationship of PDGF to tumor aggressiveness in other cancers, led to this investigation.

Our results demonstrate an association between elevated levels of PDGF in the plasma and the presence of head and neck cancer. However, there was the unexpected finding of detectable low levels of PDGF in the plasma of normal patients, which contrasted with previous reports that PDGF levels are not detectable in normals.[5] Additionally, in comparison to our control group, not all cancer patients had elevated levels. Therefore, given the limited cohort tested, the value of PDGF as a screening test using current technology cannot be supported. Of note is that 3 of the 4 patients who did not have elevated PDGF levels had undergone either radiotherapy or immunosuppressive therapy. Whether this was due to an effect of the radiation and immunosuppressants on the tumor cells or to a blunting of a response of the surrounding cells to tumor will require further investigation.

A disappointment was that PDGF levels failed to correlate with tumor stage, suggesting that PDGF may not be of value in predicting tumor aggressiveness. This is in sharp contrast to the work in breast cancer, where the correlation was apparent. However, our current staging system is imprecise, and only further follow-up of these patients will reveal the meaning of these results.

The most interesting finding, however, is that PDGF levels can potentially be used to monitor for treatment efficacy and possibly be used to identify tumor recurrence. In the 5 patients with elevated levels prior to surgery, PDGF significantly decreased after treatment. Further evaluation of a larger group of patients is ongoing to determine if this finding is consistent and, of course, whether the level increases with tumor recurrence. This is an important and exciting finding with significant clinical relevance.

It is not clear from current studies if the PDGF measured systemically is secreted from the tumor or is induced in surrounding cells. In addition, its exact role in carcinogenesis is unclear; ie, does PDGF have its major effects on the tumor cells, in an autocrine fashion, or on the surrounding cells, exerting a paracrine control over the cancer milieu? It is known to be angiogenic,[8] and may thereby promote tumor growth and metastasis. Anti-angiogenic factors are currently being investigated,[9] and reduction of plasma growth factor levels may be a method of monitoring these new therapies.

This preliminary study has demonstrated a correlation between elevated plasma PDGF level and squamous cell carcinoma of the upper aerodigestive tract. Further studies are needed to determine the utility of this as a potential biomarker in the management of head and neck cancer.

REFERENCES

1. Ross R. Platelet-derived growth factor. Annu Rev Med 1987;38:71-9.

2. Tsuda T, Yoshida K, Tsujino T, Nakayama H, Kajiyama G, Tahara E. Coexpression of platelet-derived growth factor (PDGF) A-chain and PDGF receptor genes in human gastric carcinomas. Jpn J Cancer Res 1989;80:813-7.

3. Thomas GR, Tubb EE, Sessions RB, Cullen KJ. Growth factor mRNA expression in human head and neck malignant tumors. Arch Otolaryngol Head Neck Surg 1993;119:1247-52.

4. Yoshida K, Kuniyasu H, Yasui W, Kitadai Y, Toge T, Tahara E. Expression of growth factors and their receptors in human esophageal carcinomas: regulation of expression by epidermal growth factor and transforming growth factor α. J Cancer Res Clin Oncol 1993;119:401-7.

5. Ariad S, Seymour L, Bezwoda WR. Platelet-derived growth factor (PDGF) in plasma of breast cancer patients: correlation with stage and rate of progression. Breast Cancer Res Treat 1991;20:11-7.

6. Seymour L, Dajee D, Bezwoda WR. Tissue platelet derived-growth factor (PDGF) predicts for shortened survival and treatment failure in advanced breast cancer. Breast Cancer Res Treat 1993;26:247-52.

7. Henriksen R, Funa K, Wilander E, Backstrom T, Ridderheim M, Oberg K. Expression and prognostic significance of platelet-derived growth factor and its receptors in epithelial ovarian neoplasms. Cancer Res 1993;53:4550-4.

8. McDonald NJ, Steeg PS. Molecular basis of tumour metastasis. Cancer Surv 1993;16:175-99.

9. O'Reilly MS, Holmgren L, Shing Y, et al. Angiostatin: a novel angiogenesis inhibitor that mediates the suppression of metastases by a Lewis lung carcinoma. Cell 1994;79:315-28.

Ann Otol Rhinol Laryngol 105:1996

NUCLEAR DNA CONTENT OF THE TALL CELL VARIANT OF PAPILLARY CARCINOMA OF THE THYROID GLAND

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Tall cell carcinoma of the thyroid gland is an aggressive variant of papillary carcinoma. A high nuclear DNA content has been associated with aggressive clinical behavior and an unfavorable prognosis in several malignant human tumors. It was also described in differentiated thyroid carcinoma. Therefore, analysis of the nuclear DNA content may yield information predictive of aggressive behavior. Accordingly, the DNA content of the tall cell variant was measured and compared with that of the usual form of papillary carcinoma by flow cytometry. Although all the aneuploid tumors were in the tall cell variant group, the difference in nuclear content was not statistically significant. We conclude that differences in the clinical behavior of these neoplasms are not related to alterations in DNA ploidy.

KEY WORDS — DNA content, flow cytometry, papillary thyroid carcinoma, tall cell carcinoma.

Papillary carcinoma is the most common type of cancer of the thyroid gland. It manifests a histomorphologic heterogeneity, so that several variants are recognized. Some of these variants are associated with significant prognostic implications and are therefore clinically important. The usual form of papillary carcinoma generally follows a relatively indolent clinical course and has a good prognosis. However, the tall cell variant (TCV) of papillary thyroid carcinoma is associated with more aggressive behavior and has a poor prognosis.[1-3]

Nuclear DNA analysis of some neoplasms has been shown to yield information that may predict clinical behavior and prognosis.[4] In several malignant human carcinomas, aneuploid tumors have been found to be associated with aggressive behavior and an unfavorable prognosis as compared with diploid tumors.[5] This has been described in differentiated thyroid carcinoma as well.[6] However, the diagnostic and prognostic importance of cytometric measurements of DNA for thyroid neoplasms is controversial, because some investigators have demonstrated a normal (diploid) DNA content in a variety of malignant thyroid neoplasms.[7] Therefore, the true significance of nuclear DNA content in thyroid cancer has not been fully determined.

The purpose of the present study was to determine whether nuclear DNA ploidy differs between the TCV and the usual form of papillary thyroid carcinoma and if it could serve as a marker for aggressive clinical behavior.

MATERIALS AND METHODS

The original pathology reports of all patients with papillary thyroid carcinoma surgically treated at Beilinson Medical Center between 1985 and 1994 were retrieved. A total of 238 sets of original slides were examined. The criteria for a diagnosis of the TCV were 1) cells at least twice as tall as they were wide, 2) abundant eosinophilic or oxyphilic cytoplasm, and 3) basal orientation of nuclei.[3] At least 30% of the specimen had to fulfill these criteria for the tumor to be regarded as the TCV.[3,8]

The control group, matched for sex and age, was selected by random sampling from all patients with the common form of papillary thyroid carcinoma.

Flow Cytometry. A DNA content analysis was performed with paraffin-embedded archival tissue. One 5-µm section was cut and stained with hematoxylin-eosin for light microscopy examination in order to confirm that tumor tissue was being analyzed and that the material also contained a minimum of 5% histologically benign cells to serve as diploid reference cells. Three 40-µm sections were then cut immediately adjacent to this section. The sections were cleaned of paraffin with xylene, rehydrated in a series of decreasing concentrations of ethanol, resuspended in 0.5% pepsin solution for 60 minutes at 37°C, stained with propidium iodide, and filtered through a

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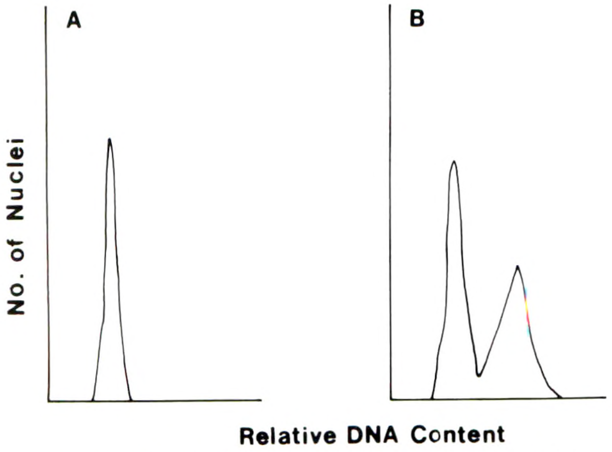

Representative DNA histograms of A) diploid and B) aneuploid tumors.

30-mm nylon mesh filter. Within 30 minutes after staining, nuclear DNA content was measured on a FACS IV flow cytometer (Becton Dickinson, Sunnyvale, Calif) with a 5-W argon laser at a wavelength of 488 nm. Histograms of 10 × 10^3 to 20 × 10^3 nuclei for each sample were recorded at a maximal scanning flow rate of 1,000 nuclei per second. Ploidy was assessed independently by two of the authors, who were blinded to the clinical status. If only one G1 peak could be seen on the histogram, the tumor was considered to be diploid (see Figure, A). If more than one peak could be identified, the tumor was considered to be aneuploid (see Figure, B). An asymmetric G1 peak was considered to contain more than one DNA stem line and thus to be aneuploid, provided the asymmetry was clear or there was further evidence of aneuploidy from multiple analyzed sections.

Statistical Analysis. Frequency was analyzed by Fisher's exact test. All p values are two-tailed and p values less than 5% were considered significant.

RESULTS

Nineteen cases of the TCV were recognized. One was excluded from the study because the original specimen was not suitable for flow cytometry analysis. Eighteen properly matched cases with the common form of papillary carcinoma served as controls. Three specimens were studied for each case. For all samples, the coefficient of variation averaged 5%. Thirty-three tumors expressed a diploid DNA content, and three tumors an aneuploid content. All the aneuploid tumors were in the TCV group, while the tumors with the common form of papillary carcinoma were all diploid. However, the difference in nuclear DNA content between the tall and regular papillary neoplastic cells was not statistically significant (p = .23).

DISCUSSION

Papillary carcinoma is the most common type of malignancy of the thyroid gland and is usually associated with a good prognosis.[9] However, the TCV is regarded as a more aggressive neoplasm with a less favorable prognosis.[1,2,4,10] The aggressiveness of the carcinoma is manifested not only by local invasion and recurrences, but also in lethality. Johnson et al[2] found a significantly higher incidence of extrathyroidal extension, local recurrence, and distant metastases in the TCV than in the common form of papillary carcinomas of the thyroid. A high rate of recurrence in TCV patients over age 50 was also reported by Terry et al.[10] Moreover, in contrast to conventional papillary carcinomas, the TCV has an excessive disease-specific mortality rate of 22% to 25%.[1,2]

The reason the TCV manifests more aggressive behavior is unknown. It has been shown that the TCV is associated with high levels of type IV collagenase.[11] The presence of higher mitotic rates in the TCV is controversial.[12] A high mitotic index has been reported in columnar cell carcinoma, another aggressive version of papillary carcinoma of the thyroid.[13] Other human neoplasms demonstrating malignant characteristics have also been found to be associated with chromosomal abnormalities and higher mitotic rates.[14] Such abnormalities are assumed to be a manifestation of an alteration in nuclear DNA content. Higher than normal DNA content has apparently been directly linked to aggressive behavior in several types of human cancers.[5] Sandritter et al[15] were the first to note a significant increase in the nuclear DNA content of thyroid cancer. However, although some reports have suggested that DNA content and ploidy analysis in thyroid nodules may predict biologic behavior,[16] other studies urge caution in the interpretation of the cytophotometric measurements of DNA in nodular lesions of the thyroid.[17] Currently available data indicate that high DNA content in thyroid carcinoma is associated with more aggressive patterns of behavior and higher mortality.[18,19] Yet, Izuo et al[20] failed to demonstrate any difference in DNA content between occult sclerosing papillary carcinoma and clinically detectable cases of papillary carcinoma. In the present study, although all the aneuploid tumors were in the TCV group, we did not observe a statistically significant difference in nuclear DNA content between the TCV and the common form of papillary carcinoma. Therefore, the aggressive clinical behavior of the TCV could not be related to an alteration in DNA ploidy.

We hope that the differences in clinical behavior between the TCV and the common papillary thyroid carcinomas will be explained by further research on the basis of other characteristics.

REFERENCES

1. Hawk WA, Hazard JB. The many appearances of papillary carcinoma of the thyroid. Cleve Clin Q 1976;43:207-16.
2. Johnson TL, Lloyd RV, Thompson NW, Beierwaltes WH, Sisson JC. Prognostic implications of the tall cell variant of papillary thyroid carcinoma. Am J Surg Pathol 1988;12:22-7.
3. Hicks MJ, Batsakis JG. Tall cell carcinoma of the thyroid gland. Ann Otol Rhinol Laryngol 1993;102:402-3.
4. Koss LG, Greenebaum E. Measuring DNA in human cancer. JAMA 1986;255:3158-9.
5. Friedlander ML, Hedley DW, Taylor IW. Clinical and biological significance of aneuploidy in human tumors. J Clin Pathol 1984;37:961-74.
6. Joensulu H, Klemi P, Eerola E, Tuominen J. Influence of cellular DNA content on survival in differentiated thyroid cancer. Cancer 1984;58:2462-7.
7. Johannessen JV, Sorbrinho-Simoes M, Lindeno T, Tangen KO. The diagnostic value of flow cytometric DNA measurements in selected disorders of the human thyroid. Am J Clin Pathol 1982;77:20-5.
8. Rosai J, Carcangiu ML, Delellis RA. Atlas of tumor pathology. Washington, DC: Armed Forces Institute of Pathology, 1992:114-21.
9. Tubrino M, Schlumberger M, Rougier P. Long-term results and prognostic factors in patients with differentiated thyroid carcinoma. Cancer 1985;55:794-804.
10. Terry JH, St John SA, Karlowski FJ, et al. Tall cell papillary thyroid cancer: incidence and prognosis. Am J Surg 1994;168:459-61.
11. Campo E, Merino MJ, Liotta L, Neumann R, Stetler-Stevenson W. Distribution of the 72-kd type IV collagenase in nonneoplastic and neoplastic thyroid tissue. Hum Pathol 1992;23:1395-401.
12. Sorbrinho-Simoes M, Sambado C, Nerland JM. Letter to the editor. Am J Surg Pathol 1989;13:74-80.
13. Sorbrinho-Simoes M, Nerland JM, Johannessen JV. Columnar-cell carcinoma of the thyroid. Am J Clin Pathol 1988;89:264-7.
14. Nowell PC. The clonal evolution of tumor cell populations. Science 1976;194:23-8.
15. Sandritter W, Carl M, Ritter W. Cytophotometric measurements of the DNA content of human malignant tumors by means of the Feulgen reaction. Acta Cytol 1966;10:26-30.
16. Kraemer BB, Srigley JR, Batsakis JG, Silva EG, Goepfert H. DNA flow cytometry of thyroid neoplasms. Arch Otolaryngol 1985;111:34-8.
17. Haemnerli G, Strauli P, Schluter G. Deoxyribonucleic acid measurements on nodular lesions of the human thyroid. Lab Invest 1968;18:675-80.
18. Cohn K, Backdahl M, Forslund G, et al. Prognostic value of nuclear DNA content in papillary thyroid carcinoma. World J Surg 1984;8:474-80.
19. Backdahl M, Corstensen J, Auer G, Tallroth E. Statistical evaluation of the prognostic value of nuclear DNA content in papillary follicular and medullary thyroid tumors. World J Surg 1986;10:974-80.
20. Izuo M, Okagaki T, Richart RM, Lattes R. Nuclear DNA content of occult sclerosing and frank papillary carcinoma of the thyroid. Cancer 1971;27:902-9.

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KINEMATIC AND DYNAMIC CHARACTERISTICS OF SOLID PELLET MOVEMENT DURING THE PHARYNGEAL PHASE OF SWALLOWING

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Information on solid particle movement through the pharynx, in addition to its physiologic importance, has relevance to the swallowing of medications in pill form. The purpose of this study was to determine the kinematics of a solid particle during the oral-pharyngeal phase of swallowing. We used a concurrent manometric-videofluoroscopic technique and identified two distinct zones of increasing bolus velocity, one at the tongue base and the other at the pharyngo-upper esophageal sphincter (UES) region. Velocity decreased significantly ($p < .05$), to 9.0 ± 1.0 cm/s, while the bolus traversed the area located between the tip of the horizontal epiglottis and the pharyngeal wall. The velocity of the liquid barium bolus head was similar to that of the solid barium pellet. The average bolus tail velocity was relatively constant. The acceleration of the barium pellet was temporally associated with development of an incrementally decreasing pressure distal to the location of the pellet in the hypopharynx and across the UES. In conclusion, the kinematics of a solid particle are similar to those of the head of a liquid bolus, but both are different from bolus tail kinematics. During the pharyngeal phase of swallowing, the area located ahead of the bolus exhibits an incrementally decreasing pressure, caudally. This may facilitate bolus transport and contribute to airway protection.

KEY WORDS — dynamics, kinematics, pharynx, pill swallow, pressure difference, solid pellet, upper esophageal sphincter.

INTRODUCTION

Information on bolus transit during the oral-pharyngeal phase of swallowing is mainly derived from studying radiopaque liquid barium boluses. Negligible information is available on the oral-pharyngeal transit of solid boluses. Transit of a bolus from the oral-pharyngeal cavity through the upper esophageal sphincter (UES) and into the esophagus can be studied from two perspectives: 1) dynamics, namely, studies of forces that induce bolus movement, and 2) kinematics, namely, studies of the motion of the bolus itself. Whereas ample data[1-8] are available on the "forces" that induce bolus movement, information regarding the "motion" of the bolus and, in particular, solid bolus movement is scarce. Available information on bolus kinematics generally pertains to liquid boluses.[9] Information on solid particle movement through the pharynx, in addition to its physiologic importance, has relevance to the swallowing of medications in pill form.

Furthermore, previous reports,[9-12] as well as our preliminary studies, have documented the development of a subatmospheric pressure in the pharyngoesophageal junction and the proximal esophagus prior to the arrival of the peristaltic pressure wave to these regions. The mechanism for development for this pressure decline, and its possible role in the dynamics and kinematics of the oral-pharyngeal phase of swallowing, has been the subject of speculation.[10-12]

For this reason, the aims of the present study were to 1) measure the velocity and acceleration of a solid barium particle as it traverses the pharynx into the proximal esophagus, 2) compare the velocity characteristics of solid particles to those of a liquid bolus, and 3) correlate pharyngeal and pharyngo-UES-esophageal pressure activity and other transport-related events to solid and liquid bolus movements.

METHODS

Six healthy male volunteers 18 to 40 years old and without a history of swallowing difficulty were studied by a concurrent manometric and videofluoroscopic technique. Studies were approved by the Human Research Review Committee of the Medical College of Wisconsin. Each subject fasted overnight before the study and the nasal cavity was anesthetized with 2% topical Xylocaine (Astra Pharmaceuticals, Westborough, Mass) applied by a cotton-tipped applicator just before the insertion of the manometric probe. The subjects gave informed consent before their studies and were studied in an upright, seated position.

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For recording the pharyngo-UES intraluminal pressure phenomenon, we used a solid-state transducer assembly. Two 3-site transducer probes with their pressure sensors 3 cm apart were secured side-by-side so that the manometric recording sites were separated by 1.5 cm. The frequency response of these solid-state transducers was 20,000 Hz. The transducer assembly was passed transnasally and positioned such that, at rest, the two most proximal manometric sites were in the hypopharynx, the two middle sites were in the UES high-pressure zone, and the two distal sites were in the proximal esophagus. The recording sites faced posteriorly. All pressure measurements were referenced to local atmospheric pressure. All pressure data were displayed on an 8-channel Sensormedics polygraph and concurrently recorded on a 16-channel Vetter FM tape recorder for subsequent analysis with a computerized digitization system.

The computer system for analyzing the manometric data consisted of a 386 IBM PC–compatible computer operating at 33 MHz. This system drives analog to digital (A/D) conversion hardware and software (DATAQ Instruments Inc, Toledo, Ohio). The A/D program called CODAS is a menu-driven capture and analysis package that allows for the asynchronous concurrent capture of up to 16 channels of data. The maximum sampling frequency of the system is 50 kHz, which exceeds the frequency requirements for sampling pharyngeal-UES pressure activity. The A/D conversion hardware and CODAS software allowed for capture and analysis of the 6-channel pressure transducer signals at sampling frequencies higher than the limitations of the polygraph recording pens (30 Hz), as well as analysis of the captured manometric data in time scales unattainable in a conventional polygraph paper and pen recording.

To record bolus motion during swallowing, we studied the subjects concurrently by videofluoroscopy. Subjects sat in a chair, positioned laterally to the face of the image intensifier. The subjects were instructed to hold their heads in a neutral position. Videofluoroscopic recordings were obtained at 90 keV with a 22.86-cm (9-in) image intensifier mode and appropriate collimation so that a lateral image was obtained of the posterior mouth, pharynx, and pharyngoesophageal region. Fluoroscopic images were recorded on a super VHS videocassette recorder (Panasonic AG-1960 Proline, Tokyo, Japan), which recorded 60 fields per second. The manometric and fluoroscopic recordings were synchronized by means of a specially designed timer (Thalner Electronics, Ann Arbor, Mich). This timer, the video recorder, and the polygraph were electrically connected. The timer generates two simultaneous signals. One signal provided a video-displayed time signal in hundredths of a second on the fluoroscopic images, and the other signal generated a 1-Hz, 5-V amplitude timing pulse that was recorded on the chart paper used for manometric recording. For each swallow, the first pulse marked on the manometric recording paper was noted and logged along with the elapsed tape count displayed on the videocassette recorder. The chart paper was run at a speed of 50 mm/s; thus, each millimeter on the paper represented 20 milliseconds. The timer signal also allowed synchronization of morphologic events recorded on videofluoroscopic images with the manometric events recorded on the FM tape recorder.

The computer analysis system used for analyzing the videofluoroscopic data consisted of a 486 IBM–compatible computer operating at 33 MHz. The computer system drives an A/D conversion board and program specifically designed for image capture and analysis. The image analysis and capture software (JAVA, Jandel Scientific, San Mateo, Calif) allows capture of standard raster scan video images and morphologic analysis of digitized image data. The digitized images are stored as computer files for any subsequent recall or analysis.

All six subjects were studied during four swallows of each of the following: 1) a barium pellet (0.2-cm-diameter spherical pills), 2) 5 mL liquid barium, and 3) dry swallows. For all swallows we determined the velocity and acceleration of the barium pellet and the head and tail of the barium liquid bolus as they traversed the pharynx, UES, and subsphincteric area of the esophagus. The videofluoroscopic images were digitized by the computer image analysis system at a rate of one video field every 60th of a second. The video analysis software was used to mark the x-y coordinates of the center of the pellet and boundaries of the liquid barium bolus head and tail as the bolus moved through the pharynx and UES. The time-dependent coordinates were stored by the image analysis program in spreadsheet format. Thus, for any stored set of swallow sequence images, a time-dependent (x-y) mapping of bolus head and tail location was obtained. The bolus head and tail coordinates were numerically differentiated with respect to time to yield the velocity, and differentiated twice to yield acceleration of the bolus head and tail. Along with the bolus kinematic data, we also determined, on fluoroscopy, the positions of the barium pellet and liquid barium bolus head and tail relative to the instant that the epiglottis appeared to make contact with the arytenoid cartilages at the posterior larynx (laryngeal vestibular closure), and relative to the instant of

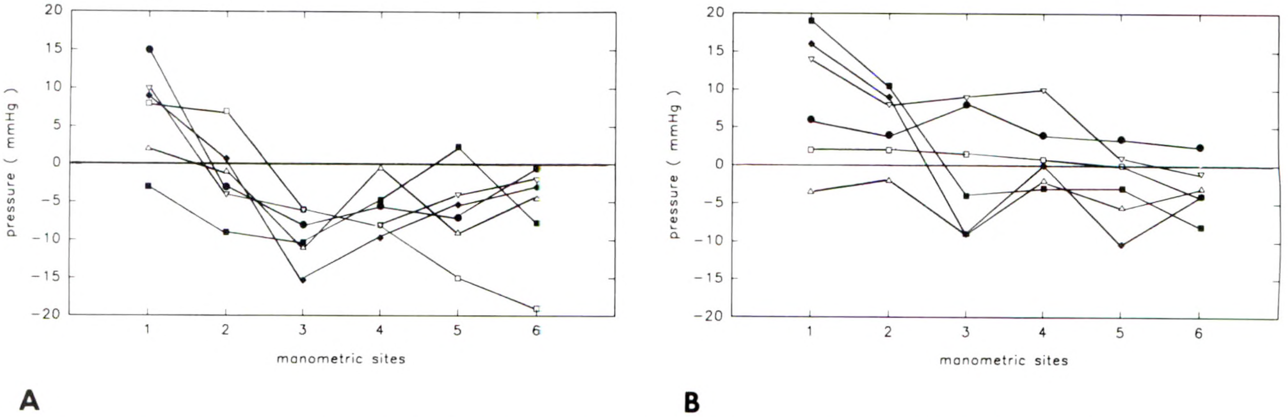

Fig 1. Pressure phenomenon in pharyngoesophageal junction immediately before onset of peristaltic pressure wave at most proximal transducer for A) pellet barium and B) liquid barium. Mean data for each subject and for each site are presented. As seen, ahead of peristaltic pressure wave there is downhill pressure gradient from pharynx to esophagus.

separation of the anterior and posterior walls of the pharyngoesophageal segment (UES opening). We determined the intraluminal pressure relative to the location of the barium pellet and liquid barium bolus head and tail. From the videofluoroscopic images, the position of the bolus relative to the manometric recording sites was determined throughout the swallow sequence. With use of the timing signal that coordinated the fluoroscopic images and manometric events for every video field, the pressure phenomenon proximal and distal to the bolus was determined for all barium pellet and barium liquid swallow sequences.

In addition, we performed the following tests to determine whether the rapidly changing negative pressure excursions recorded during swallowing in the pharyngoesophageal junction were real manometric events or transducer artifacts. We tested the hypothesis that during swallowing, when the UES wall at the instant of its opening suddenly moves away from the transducer, the transducer surface is bent outward because of surface tension until the surface tension is overcome by the opposing transducer surface force ("ringing"). The outward deflection of the transducer surface would result in a recorder signal that oscillates as the transducer surface returns to some steady state deflection. The part of the oscillations that occurs when the transducer surface position is deflected outward past the calibrated "zero" pressure transducer deflection is registered as "negative" (subatmospheric) pressure. To test this hypothesis, the "ringing" effect was studied by applying a constant surface pressure to a transducer site and suddenly releasing it. The test was performed for the following applied pressures: 10, 75, 150, and 200 mm Hg. To generate these pressures, the transducer was simply pressed against the hard bench surface by the pulp of the index finger, and when the desired pressure was reached, it was maintained for a few seconds and then the finger was suddenly removed. To simulate the damp surface of the pharynx and UES, a wet finger was placed on the transducer surface, producing a given pressure, and then suddenly released. The output of the calibrated transducer signal was recorded by a computer digitization system (described above) for subsequent analysis. The transducer output was also displayed on a Sensormedics polygraph recorder at the time of the test. To study the effect of various degrees of surface tension, besides using a damp surface, the response of the transducer was tested with a dry surface and a surface coated with Xylocaine gel. The test was also performed under 10 cm of water. Results were analyzed for wave duration and nadir pressure and were compared to subatmospheric pressures recorded from the pharyngo-UES area of our volunteers. Statistical analysis was done by means of ANOVA with repeated measures. Data in the text are presented as mean ± SD unless stated otherwise.

RESULTS

Solid Pellet and Liquid Bolus Dynamics. Analysis of the manometric recordings showed that with each swallow there was recorded a peristaltic pressure wave that propagated from the most proximal transducer site distally. Analysis of concurrent videofluoroscopic recordings showed that at the peak of deglutitive UES and laryngeal excursions, the most proximal manometric recording site was positioned within the hypopharynx in the vicinity of the laryngeal vestibule; three sites were in the pharyngoesophageal junction (90% of the total analyzed swallow sequences), and the remaining two sites were positioned in the proximal esophagus.

In addition, analysis of the pressure phenomenon within the pharynx, UES, and proximal esophagus showed that during each swallow, immediately be-

TABLE 1. MAGNITUDE AND DURATION OF SUBATMOSPHERIC PRESSURE EXCURSIONS DUE TO SOLID STATE TRANSDUCER RESPONSE TO RELEASE OF VARIOUS APPLIED PRESSURES

		Applied Pressures (mm Hg)			
		10	*75*	*150*	*200*
Pressure (mm Hg)	D	−2.60 ± 2.100	−5.30 ± 1.60	−5.60 ± 1.30	−8.30 ± 2.500
	W	−2.90 ± 1.800	−5.90 ± 1.10	−5.60 ± 1.30	−8.90 ± 2.200
	J	−2.90 ± 1.600	−7.50 ± 4.50	−9.80 ± 3.00	−10.10 ± 3.300
	U	−2.80 ± 1.600	−6.20 ± 1.50	−8.70 ± 1.40	−9.10 ± 2.000
Duration (s)	D	0.03 ± 0.003	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.003
	W	0.02 ± 0.003	0.03 ± 0.01	0.03 ± 0.01	0.03 ± 0.010
	J	0.03 ± 0.003	0.02 ± 0.01	0.03 ± 0.01	0.03 ± 0.020
	U	0.03 ± 0.010	0.03 ± 0.01	0.03 ± 0.01	0.02 ± 0.010

Values are mean ± SD.

D — dry transducer surface, W — water coating transducer surface, J — Xylocaine jelly coating transducer surface, U — transducer submerged under 10 cm of water.

fore the onset of the peristaltic pressure wave at the most proximal manometric site, there was a transient pressure decline recorded in all six transducer sites for dry swallows as well as liquid barium and barium pellet swallows.

Analysis of concurrent videofluoroscopic recordings of pellet and barium swallows showed that development of this pressure decline was concurrent with anterior displacement of the hyoid-larynx-cricoid complex and occurred in the hypopharynx, UES, and proximal esophageal recording sites located caudad to the luminal closure induced by the peristaltic wave. Distribution of this transient pressure decline, in this domain, is shown in Fig 1. In 83% of solid pellet and 55% of liquid barium swallows, the ambient pressure distal to the peristaltic pressure wave decreased to subatmospheric ("negative") pressures. The phenomenon of subatmospheric pressure was observed to various degrees in all subjects. A comparison of the "negative" pressures generated during swallowing and the "negative" pressures generated in the bench experiments by the "ringing effect" is shown in Tables 1 and 2. Representative examples of these two phenomena are shown in Fig 2. There was a direct relationship between the magnitude of applied pressure and the magnitude of "negative" pressure induced by the "ringing" phenomenon. However, duration of this "negative" pressure was not dependent on the magnitude of the applied pressure. The average wave duration found in the "ringing" tests ranged between 0.02 and 0.04 seconds. The negative (subatmospheric) pressure excursions found in volunteers in the present study were 5 to 10 times longer than those of the "ringing" ($p < .05$).

Pellet Kinematics. Analysis of the videofluoroscopic recordings showed that during each swallow, the velocity of the pellets traversing the pharynx, UES, and proximal esophagus was variable. The velocity variability was found to be location-dependent. We identified two distinct zones of increasing velocity. The average velocity of the pellet traversing the posterior aspect of the tongue was 18.0 ± 4.2 cm/s. It increased to 39.4 ± 7.6 cm/s while the pellet traversed the base of the tongue to the level of the epiglottis (supraepiglottic region). During this time the larynx had reached its maximum elevation and the epiglottis had assumed its horizontal orientation. The pellet velocity decreased significantly ($p < .05$), to 9.0 ± 1.0 cm/s, while it traversed the area located between the tip of the horizontal epiglottis and the pharyngeal wall (pharyngoepiglottic space). After the pellet passed this area, its velocity increased to an

TABLE 2. MAGNITUDE AND DURATION OF SUBATMOSPHERIC PRESSURE DECLINES DEVELOPED IN PHARYNX, UPPER ESOPHAGEAL SPHINCTER, AND PROXIMAL ESOPHAGUS OF NORMAL VOLUNTEERS DURING SWALLOWING OF LIQUID AND SOLID BARIUM BOLUSES

	Magnitude (mm Hg)		*Duration (s)*	
Site	*Pellet*	*Liquid*	*Pellet*	*Liquid*
1	−4.8 ± 2.8	None	0.24 ± 0.08	0.25 ± 0.07
2	−8.0 ± 3.9	−9.3 ± 3.1	0.28 ± 0.12	0.29 ± 0.12
3	−9.3 ± 4.1	−9.5 ± 4.8	0.44 ± 0.12	0.42 ± 0.24
4	−9.4 ± 4.2	−9.6 ± 5.0	0.43 ± 0.19	0.31 ± 0.18
5	−10.9 ± 6.3	−10.1 ± 6.0	0.44 ± 0.15	0.31 ± 0.20
6	−11.1 ± 6.6	−11.0 ± 6.3	0.49 ± 0.19	0.61 ± 0.18

Site 1 is most proximal, site 6 most distal. Values are mean ± SD.

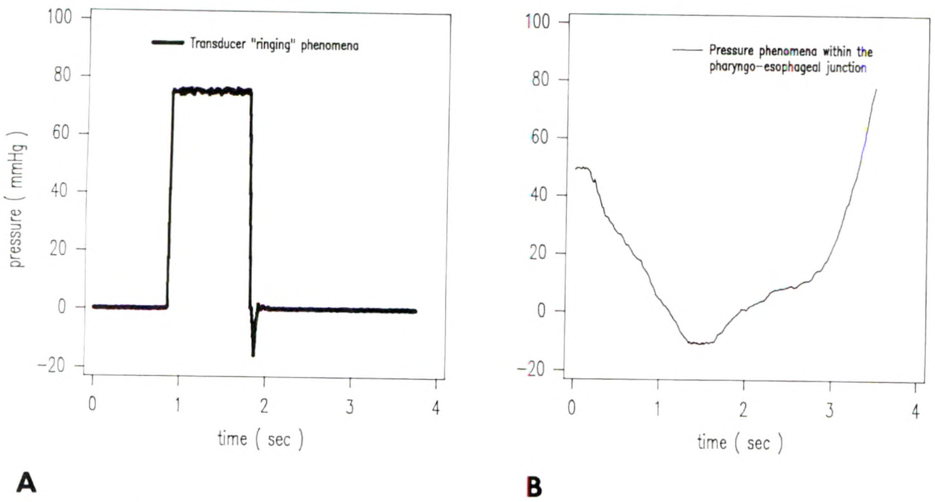

Fig 2. Examples of A) subatmospheric pressure generated by "ringing" phenomenon of strain gauge transducer following release of 78 mm Hg pressure, and B) subatmospheric pressure registered by similar transducer within pharyngoesophageal segment. Note that although magnitudes of negative pressure in both instances are similar, duration of subatmospheric pressure in pharyngoesophageal segment is significantly longer.

average of 32.0 ± 11.1 cm/s while it passed through the pharyngo-UES area to enter the proximal esophagus ($p < .05$). Following entry into the esophagus, pellet velocity decreased to 11.3 ± 1.9 cm/s ($p < .05$). This proximal esophageal pellet velocity was measured in 16 instances that the pellet could reliably be seen moving through the proximal esophagus. In the rest, the pellet stayed in the subsphincteric region of the esophagus until it was cleared by a subsequent water swallow. A composite time history of average pellet velocity is shown in Fig 3. In 10 instances, the barium pellet was seen to halt for 0.05 ± 0.02 second near the UES proximal margin before it accelerated to the above-mentioned velocity.

Liquid Bolus Kinematics. Videofluoroscopic analysis of liquid bolus kinematics showed that during each swallow the kinematic behavior of the head of the liquid barium bolus was significantly different from that of the tail of the bolus. The velocity of the liquid barium bolus head showed the same two zones of acceleration reported for the solid barium pellet. The anatomic locations of the two acceleration zones were identical. The average bolus head velocity over the posterior aspect of the tongue was 19.5 ± 4.0 cm/s. At the supraepiglottic region, the bolus head velocity was 39.0 ± 8.0 cm/s. The bolus head decelerated to 18.6 ± 8.6 cm/s at the pharyngoepiglottic space before accelerating to 36.7 ± 12.4 cm/s in the infraepiglottic region. The bolus head velocity was not significantly different from the pellet velocity in the regions described above. The average bolus tail velocity as it traversed the pharynx and passed through the UES was relatively constant at 10.3 ± 3.0 cm/s (Fig 3).

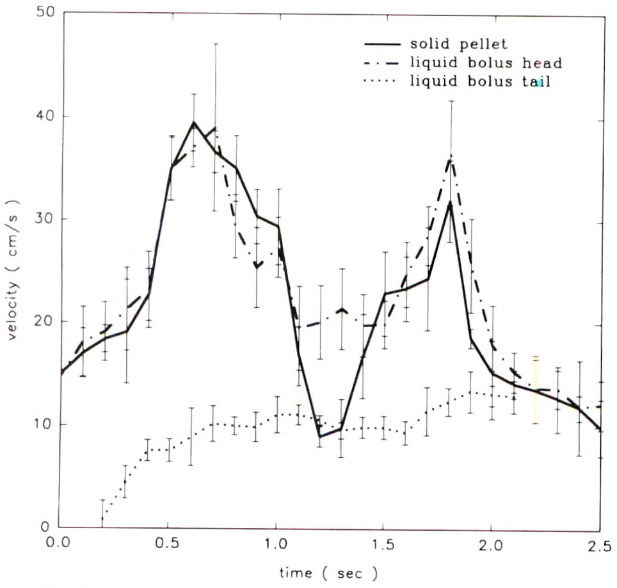

Fig 3. Composite time history of average velocity of solid pellet, liquid barium bolus head, and tail traversing pharynx. As seen, velocity and acceleration of barium pellet and liquid barium bolus head were similar. However, both were significantly different from those of liquid barium bolus tail.

Pellet Kinematic and Dynamic Interrelationship. Analysis of the concurrent manometric and videofluoroscopic recordings showed that the pellet traveled ahead of the peristaltic pressure wave. The distance between the pellet and the peristaltic front was 1.4 ± 0.2 cm when the peristaltic contraction wave reached the most proximal manometric site in the pharynx. The acceleration of the barium pellet was associated with incrementally decreasing pressure distal to the location of the pellet in the hypopharynx and across the UES. As the pellet passed each manometric recording site, the concurrent pressure at each site distal to it showed a progressive decline (Fig 4).

Liquid Bolus Kinematic and Dynamic Interrelationship. Analysis of concurrent manometric and videofluoroscopic recordings showed that the kinematic and dynamic characteristics of the head of the bolus were significantly different from those of the bolus tail. As with the pellet swallows, there was an

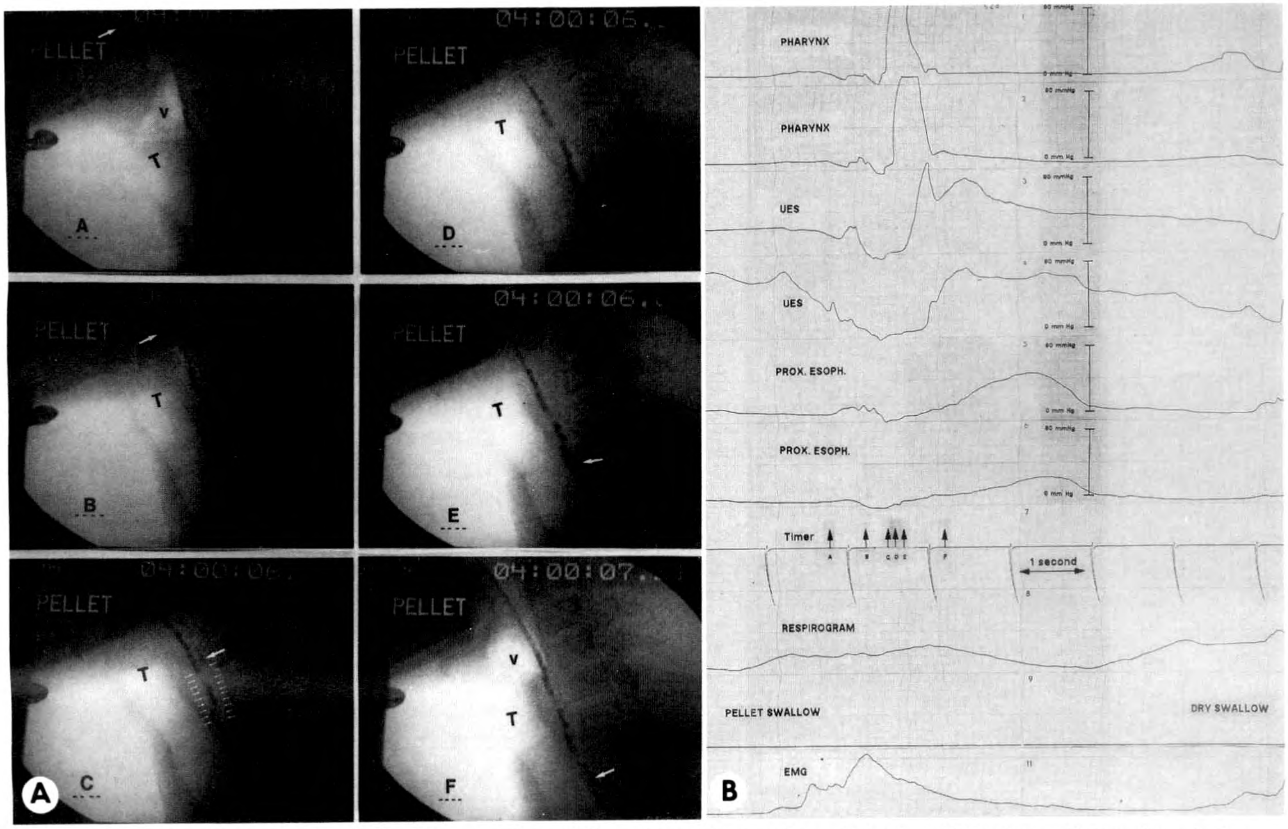

Fig 4. Example of concurrent A) videofluoroscopic and B) manometric recordings of single solid barium pellet swallow during pharyngeal phase of swallowing. Letters A-F marked by arrow on manometric recording correspond temporally with panels A-F on fluoroscopic recording. As seen at rest on panel A of fluoroscopic recording, two most proximal transducers are at level of laryngeal vestibule (v). Proximal end of tracheal air column (T) corresponds to level of vocal cords. At each level, pellet marked by thick white arrow is accompanied by subatmospheric pressure gradient distal to it. At instant that is marked by arrow C on manometric recording, pressure transducers are seen fluoroscopically to be located ahead of bolus within open chamber filled with air (prebolus zone) during opening of upper esophageal sphincter (rows of thin arrows), and transducers measure subatmospheric pressure. Pellet is seen immediately proximal to this zone.

incrementally decreasing pressure distribution distal to the bolus head. The temporal and spatial pressure distributions in the regions located distal to the head of the barium bolus were similar to those for pellet swallows (Fig 3).

The forces related to bolus tail kinematics were associated with the peristaltic pressure wave. Comparison of the peristaltic pressure wave velocity, measured manometrically, and the bolus tail speed, measured fluoroscopically, showed that the two phenomena are virtually identical. Also, analysis of the concurrent manometric and fluoroscopic recordings showed that the upstroke of the peristaltic pressure wave was always associated temporally and spatially with the tail of the barium bolus. In addition, analysis of concurrent manometric and fluoroscopic recordings identified three different zones in relation to the position of the bolus: 1) the peristaltic zone, located behind the bolus, 2) the bolus zone, occupied by the bolus, and 3) the prebolus zone, ie, the area located ahead of the bolus. In the peristaltic zone, the lumen is occluded by muscular contraction. The bolus zone is the unclosed luminal region that includes the bolus. Movement of the bolus in the bolus zone is partly a direct result of local lumen contraction and partly a result of any inertia-related dynamics that took place in the peristaltic zone. (In our study, subjects were in an upright position, so gravity could also be considered partly responsible for bolus movement.) The prebolus zone is the region immediately distal to the position of the bolus (Fig 5). The pressure in the prebolus zone was always less than the pressure in the bolus zone. The magnitude of pressure in a part of the prebolus zone often was subatmospheric. Thus, dynamic pressure activity in the prebolus zone created a pressure difference between the bolus zone and the prebolus zone, further affecting bolus behavior.

DISCUSSION

In this study, we determined the kinematic and dynamic characteristics of a solid pellet during the pharyngeal phase of swallowing, and compared the findings with those of liquid bolus swallows. The main findings of the study were that 1) solid bolus

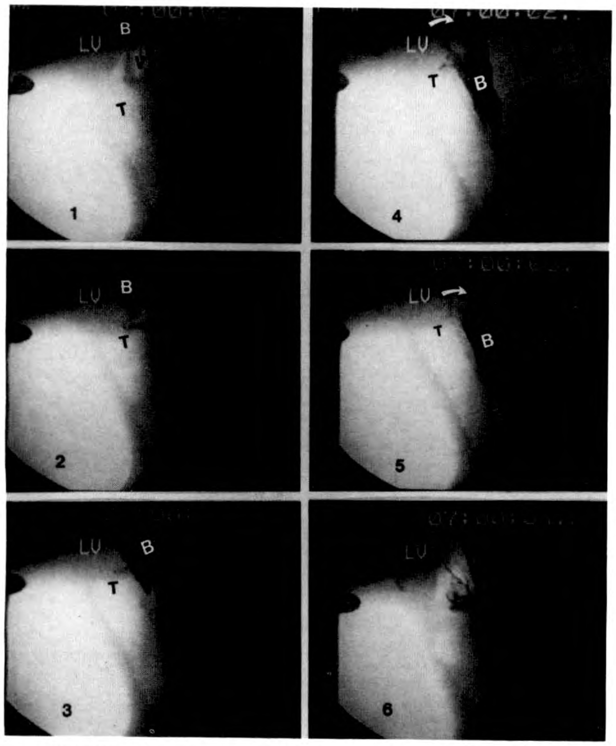

Fig 5. Still frames of videofluoroscopic recording of 5-mL barium swallow during pharyngeal phase of deglutition demonstrating peristaltic, bolus, and prebolus zones. LV — laryngeal vestibule. 1) Barium bolus (B) has just entered pharynx. Vestibule (V) is partially closed. Upper border of tracheal air column (T) corresponding to vocal cords is shown. 2,3) Barium has progressed into pharynx 0.07 and 0.17 seconds later. Laryngeal vestibule is now completely closed and larynx is displaced anteriorly. 4) Barium bolus has progressed further. Swallowed air is seen traversing ahead of barium, while luminal closure (arrow) follows barium. 5) Peristaltic zone resulting in luminal closure (curved arrow), bolus zone filled with liquid barium (B), and prebolus zone (parallel rows of thin arrows) are better seen than in panel 4. 6) Laryngeal vestibule has now reopened. Swallow has ended and larynx returned to its original position.

kinematics are similar to the kinematics of the head of a liquid bolus; 2) from a kinematic point of view, the head of a liquid bolus behaves differently from the tail of the bolus; 3) the velocity characteristics of the tail of a liquid bolus are similar to those of pharyngeal peristalsis, and both have constant velocity; and 4) during swallowing, associated with the anterior movement of the larynx and UES opening, a pressure gradient develops in the area of the hypopharynx, UES, and proximal esophagus, prior to the arrival of the bolus. In this region, the more distal sites exhibit a lower pressure compared to the proximal sites. Frequently, these pressures are subatmospheric. The duration and magnitude of these recorded subatmospheric pressures are significantly different from those induced by transducer "ringing."

Viewed from a dynamic perspective, our study findings indicate that at any instant during the pharyngeal phase of swallowing three distinct dynamic pressure zones exist within the anatomic region extending from the hypopharynx to the proximal esophagus: 1) the peristaltic zone, which fluoroscopically corresponds to the region of luminal closure, 2) the bolus zone, which fluoroscopically corresponds to the open lumen that is occupied by the bolus, and 3) most distally, the prebolus zone, which fluoroscopically corresponds to the open lumen that is occupied by air or liquid mist. The pressure phenomena in the pharyngeal peristaltic and bolus zones have been reported previously.[6-8] The findings of the present study show that within the prebolus zone there exists an incremently decreasing pressure distribution across the UES such that the proximal esophagus exhibits a lower pressure compared to the distal pharynx. These pressures frequently reach subatmospheric range. These pressure declines were found to be temporally associated with deglutitive anterior displacement of the cricolaryngohyoid complex.

In addition, during swallowing and associated with anterior-superior laryngeal movement and UES opening when the epiglottis has reached its tilted position covering the glottis, there exist two zones of bolus acceleration located, respectively, at the supraepiglottic and infraepiglottic regions. The velocity and acceleration of the bolus in the supraepiglottic and infraepiglottic zones are similar. However, these zones are separated by a region of bolus deceleration located between the edge of the tilted epiglottis and the posterior pharyngeal wall: the pharyngoepiglottal space.

To our knowledge, the kinematics of a solid bolus during the pharyngeal phase of swallowing have not been reported previously. However, these findings corroborate an earlier report[9] regarding the kinematics of the head of a liquid bolus during the oral-pharyngeal phase of swallowing. Contrary to this report, in the present study we did not find any acceleration of the tail of the barium bolus during the pharyngeal phase of swallowing.

The instances of maximum pellet and liquid bolus head acceleration across the UES were always closely associated with maximum pressure differences between the pressure site immediately distal to the bolus position and the pressure in the proximal esophagus. However, a direct cause-and-effect relationship between these two phenomena cannot be ascertained from the findings of this study. We may, however, speculate that this pressure drop provides a preferential path to the bolus away from the airway by facilitating its transport across the UES and into the esophagus. In this context, this pressure drop, in addition to its possible role in bolus transport, may act as an airway protective mechanism.

Our study findings indicate the existence of two distinct modes of transport for solid barium pellet as well as liquid barium boluses. One mode of transport is characterized by rapid acceleration of material across the base of the tongue and through the UES. This mode is temporally associated with the transsphincteric pressure drop as well as with the forces generated by rapid posterior movement of the tongue base, seen on fluoroscopy. The second mode of transport is characterized by a slower clearance of material from the oropharynx associated with the oropharyngeal peristaltic pressure wave. These modes of transport appear to be secondary to the structural and kinematic events that occur in the pharynx and pharyngoesophageal segment.

Barclay,[10] in 1930, postulated from high-speed cineradiographic recordings of passage of food through the pharynx the presence of a short-duration "negative" pressure in the pharynx "sucking the food down." He reported a subatmospheric pressure of 14 to 16 inches of water (26 to 36 mm Hg) in the pharyngo-UES segment during swallowing. Concurrent fluorographic and manometric analyses of liquid barium swallows have been reported in the literature using experimental techniques similar to those of the present study; however, while the phenomenon of subatmospheric ("negative") pressures has been reported in some studies,[10-13] in others, this phenomenon has not been discussed. These earlier videofluoroscopic and manometric assessments of swallowing have used two or four widely spaced manometric recording sites spanning the hypopharynx, pharynx, UES, and proximal esophagus. Such a limited number of widely spaced recording sites compromises the accuracy of dynamic evaluation of transpharyngeal pressure activity, since catheter movement during swallowing could temporarily shift any single probe out of the region that exhibits subatmospheric pressure.

The phenomenon of "negative" pressures is further complicated by the fact that in a few published reports the duration of some of these "negative" pressure excursions — although not reported directly as such — when measured from the presented figures, occur in time intervals that are consistent with the solid state strain gauge "ringing" effects reported in the present paper. According to the findings of the present study, both physiologic and "ringing"-induced negative pressure excursions may occur during swallowing. Differences in recording sites and techniques may explain these discrepancies.

In summary, the kinematics of a solid particle are similar to those of the head of a liquid bolus, but both are different from bolus tail kinematics. During the pharyngeal phase of swallowing there exist three distinct manometric zones in the pharyngoesophageal region. These are the peristaltic zone, bolus zone, and prebolus zone. The pressure signature of each zone is unique. The prebolus zone exhibits a positive pressure difference that may facilitate bolus transport through the UES and, by doing so, contribute to airway protection. Two distinct modes of transport are evident during the pharyngeal phase of swallowing, one mode being characterized by rapid movement associated with inertial and dynamic effects of tongue thrust proximal to swallowed material and variable positive pressure differences distal to it, and a second mode being characterized by slow movement associated with peristalsis.

REFERENCES

1. Shaker R, Cook IJS, Dodds WJ, Hogan WJ. Pressure-flow dynamics of the oral phase of swallowing. Dysphagia 1988;3:79-84.

2. Dodds WJ, Hogan WJ, Lydon SB, Stewart ET, Steff JJ, Arndorfer RC. Quantitation of pharyngeal motor function in normal human subjects. J Appl Physiol 1975;39:692-6.

3. Castell JA, Boag Dalton C, Castell DO. Pharyngeal and upper esophageal sphincter manometry in humans. Am J Physiol 1990;258(Gastrointest Liver Physiol 21):G173-G178.

4. Sears VW, Castell JA, Castell DO. Radial and longitudinal asymmetry of human pharyngeal pressures during swallowing. Gastroenterology 1991;101:1559-63.

5. Pryde A, Wilson JA, Macintyre CCA, Heading RC. Radial and axial asymmetry of the pharynx. J Gastrointest Motility 1991;3:196.

6. Shaker R, Ren J, Podvrsan B, et al. Effect of aging and bolus variables on pharyngeal and upper esophageal sphincter motor function. Am J Physiol 1993;264(Gastrointest Liver Physiol 27):G427-G432.

7. Robbins J, Hamilton JW, Lof GL, Kempster GB. Oropharyngeal swallowing in normal adults of different ages. Gastroenterology 1992;103:823-9.

8. Perlman AL, Guthmiller Schultz J, VanDaele DJ. Effects of age, gender, bolus volume, and bolus viscosity on oropharyngeal pressure during swallowing. J Appl Physiol 1993;75:33-7.

9. Ku DN, Ma P, McConnel FMS, Cerenko D. A kinematic study of the oropharyngeal swallowing of a liquid. Ann Biomed Eng 1990;18:655-69.

10. Barclay AE. The normal mechanisms of swallowing. Proc Staff Meeting Mayo Clin 1930;5:251-7.

11. McConnel FMS. Analysis of pressure generation and bolus transit during swallowing. Laryngoscope 1988;98:71-8.

12. McConnel FMS, Cerenko D, Jackson RT, Guffin TN. Timing of major events of pharyngeal swallowing. Arch Otolaryngol Head Neck Surg 1988;114:1413-8.

13. McConnel FMS, Cerenko D, Mendelson M. Manofluorographic analysis of swallowing. Otolaryngol Clin North Am 1988;21:625-35.

Ann Otol Rhinol Laryngol 105:1996

CAUSE OF EUSTACHIAN TUBE CONSTRICTION DURING SWALLOWING IN PATIENTS WITH OTITIS MEDIA WITH EFFUSION

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In order to clarify the cause of the constriction of the eustachian tube during swallowing that is often seen in patients with otitis media with effusion, video endoscopy of the pharyngeal orifice of the eustachian tube was performed and superimposed with videograms of the tubal airflow and resistance, which were simultaneously examined by the forced response test. In children with otitis media with effusion (17 ears), when the eustachian tube constricted on the videogram on swallowing, the tubal orifice was found to be squeezed between an elevated soft palate and a hypertrophied adenoid (7/17), squeezed between an elevated soft palate and edema of the posterior lip (7/17), or blocked by nasal discharge (5/17). In adults with otitis media with effusion (7 ears), edema of the posterior lip (5/7) was the main cause of the constriction of the tubal orifice during swallowing. Inflammation in the nasopharynx and the pharyngeal portion of the eustachian tube was considered to be closely related to the tubal constriction, which represents a considerable part of the cause of tubal ventilatory dysfunction in otitis media with effusion.

KEY WORDS — endoscopy, eustachian tube dysfunction, forced response test, otitis media with effusion.

INTRODUCTION

It is well known that eustachian tube (ET) dysfunction, especially impairment of its active ventilatory function, is closely related to the pathogenesis of otitis media with effusion (OME).[1-3] Since the forced response test,[4] with which behaviors of the ET during various tasks like swallowing can be detected aerodynamically, came into clinical use, it has been found that approximately 40% to 80% of the ETs of children with OME showed constriction during swallowing, in contrast with dilation in normal subjects.[5,6] Furthermore, our previous aerodynamic study of the ET revealed that the site of ventilatory dysfunction lay near the pharyngeal end of the ET in many of the patients with OME.[7] An important question to be solved now is what happens to the pharyngeal portion of the ET of patients with OME during swallowing.

In this study, an attempt was made to clarify the cause of ET constriction during swallowing in those patients by using videoendoscopy of the pharyngeal orifice of the ET superimposed with videograms of the tubal airflow and resistance examined by the forced response test.

MATERIALS AND METHODS

Included in this study were 17 ears of 17 children (6 to 14 years old) with OME, 7 ears of 7 adults (52 to 69 years old) with OME, and an adult with a traumatic perforation of the tympanic membrane with no history of any otological disease as a control. The patients with OME had been treated with ventilation tubes, and their ETs were found to constrict during swallowing by the forced response test.[4]

During the forced response test, the pharyngeal orifice of the ET was examined with a flexible endoscope (Olympus, ENF type P or L) connected to a portable video camera (Nagashima CCD camera, Tokyo, Japan). The airflow rate (20 mL/min) through the ET and airflow resistance (in millimeters of water per milliliter per minute) of the ET were superimposed on the monitor by means of a videogram (Hamamatsu TV Ltd, FOR.A, VG-40, Hamamatsu, Japan). Thus, both the movement of the ET and changes of the airflow and resistance of the ET during swallowing were recorded simultaneously, and were analyzed frame by frame. The monitoring was usually continued for several minutes with several swallows by the patients to obtain consistent findings. In an adult patient with OME (case 2), an inflation catheter was inserted into the tubal orifice after the above-mentioned routine examination, and the change in the behavior of the ET was examined.

RESULTS

A control and 3 representative cases are presented.

Case 1. A 22-year-old man (control, Fig 1) had a tubal pharyngeal orifice that was clear and open to the nasopharynx at rest. During swallowing, his pharyngeal orifice remained open when the airflow increased and the resistance decreased, indicating that his ET dilated. After swallowing, both the airflow

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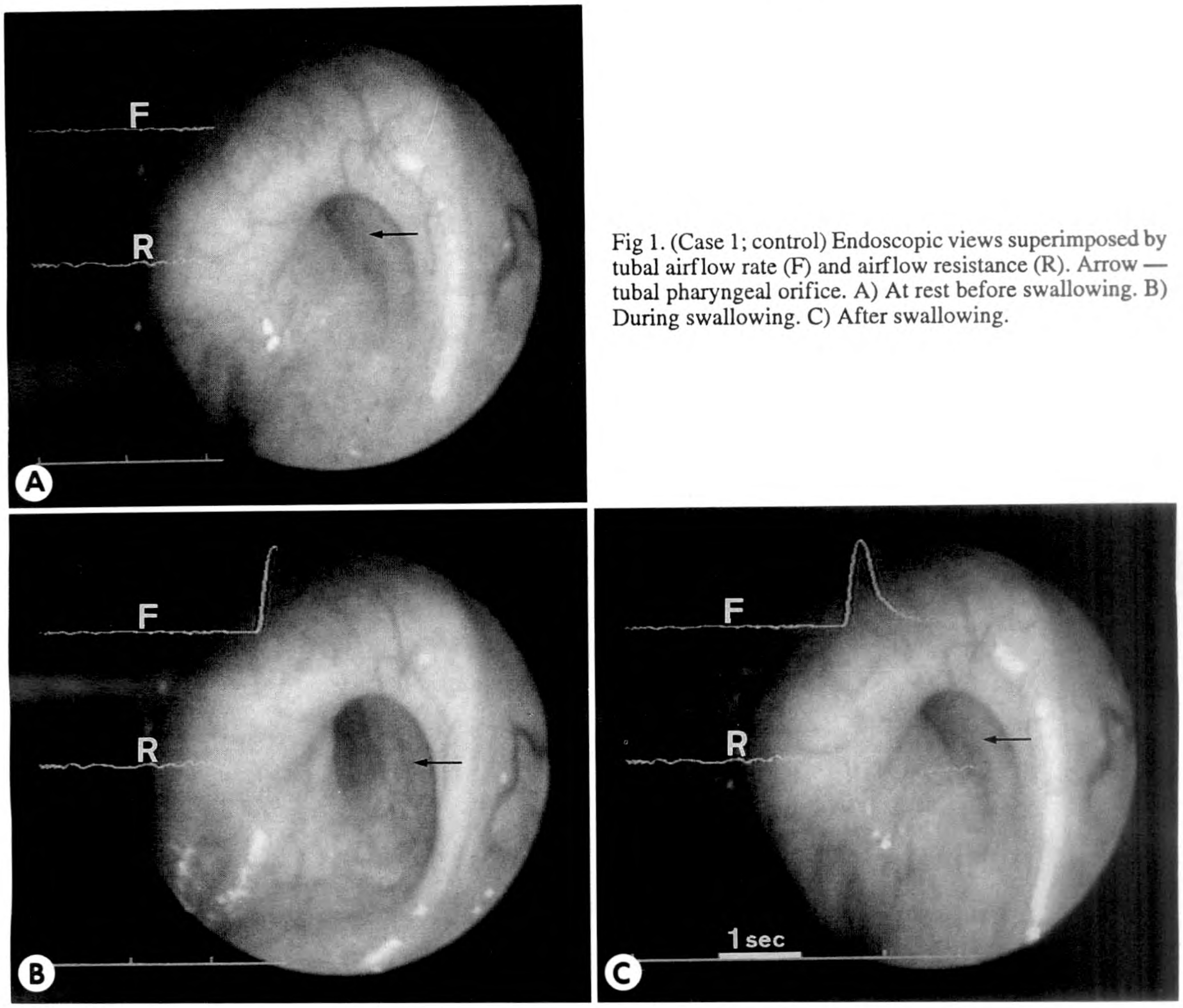

Fig 1. (Case 1; control) Endoscopic views superimposed by tubal airflow rate (F) and airflow resistance (R). Arrow — tubal pharyngeal orifice. A) At rest before swallowing. B) During swallowing. C) After swallowing.

and resistance returned to the original levels.

Case 2. A 52-year-old man with OME (Fig 2) had a tubal pharyngeal orifice that showed edema, especially at its posterior lip. When he swallowed and the airflow decreased and resistance increased, the pharyngeal orifice was squeezed by the elevated soft palate and the edematous posterior lip of the ET. This constriction, however, did not occur when a tubal inflation catheter was inserted into his tubal pharyngeal orifice approximately 1 cm in depth to keep the orifice open; this demonstrated that the ET constricted at its pharyngeal orifice on swallowing.

Case 3. A 5-year-old girl with OME (Fig 3) had a hypertrophied adenoid that was observed just medial to the posterior lip of the ET on endoscopy. As her soft palate was elevated on swallowing, her tubal pharyngeal orifice was found to be squeezed by the elevated soft palate and the posterior lip of the ET, because the hypertrophied adenoid prevented the posterior lip from being elevated together with the soft palate. As a result, the airflow decreased and the airflow resistance increased, indicating constriction of the ET at its pharyngeal orifice. After the swallow, her tubal pharyngeal orifice was open, and the airflow and resistance returned to the original level.

Case 4. A 64-year-old woman with OME (Fig 4) had paranasal sinusitis and edema around her tubal pharyngeal orifice. When she swallowed, the airflow decreased and the resistance increased, indicating tubal constriction. After medical treatment of her sinusitis for several months, the edema of her tubal pharyngeal orifice was found to be improved and the airflow increased and the resistance decreased during swallowing, indicating that her ET dilated.

Among 17 ears of children, a hypertrophied adenoid (7 ears, 41%) and edema of the pharyngeal orifice (7 ears, 41%) were the most frequent causes of constriction, followed by blockage of the tubal pharyngeal orifice by nasal discharge (5 ears, 29%) and a hypertrophied peritubal tonsil (1 ear, 6%). These causes overlapped in 4 ears: both a hypertrophied adenoid and nasal discharge were observed in 3 ears, and both edema and nasal discharge in 1 ear. In a 13-year-old girl, the cause of the tubal constriction was unknown in the present study, since her pharyngeal orifice was observed by endoscopy to open during

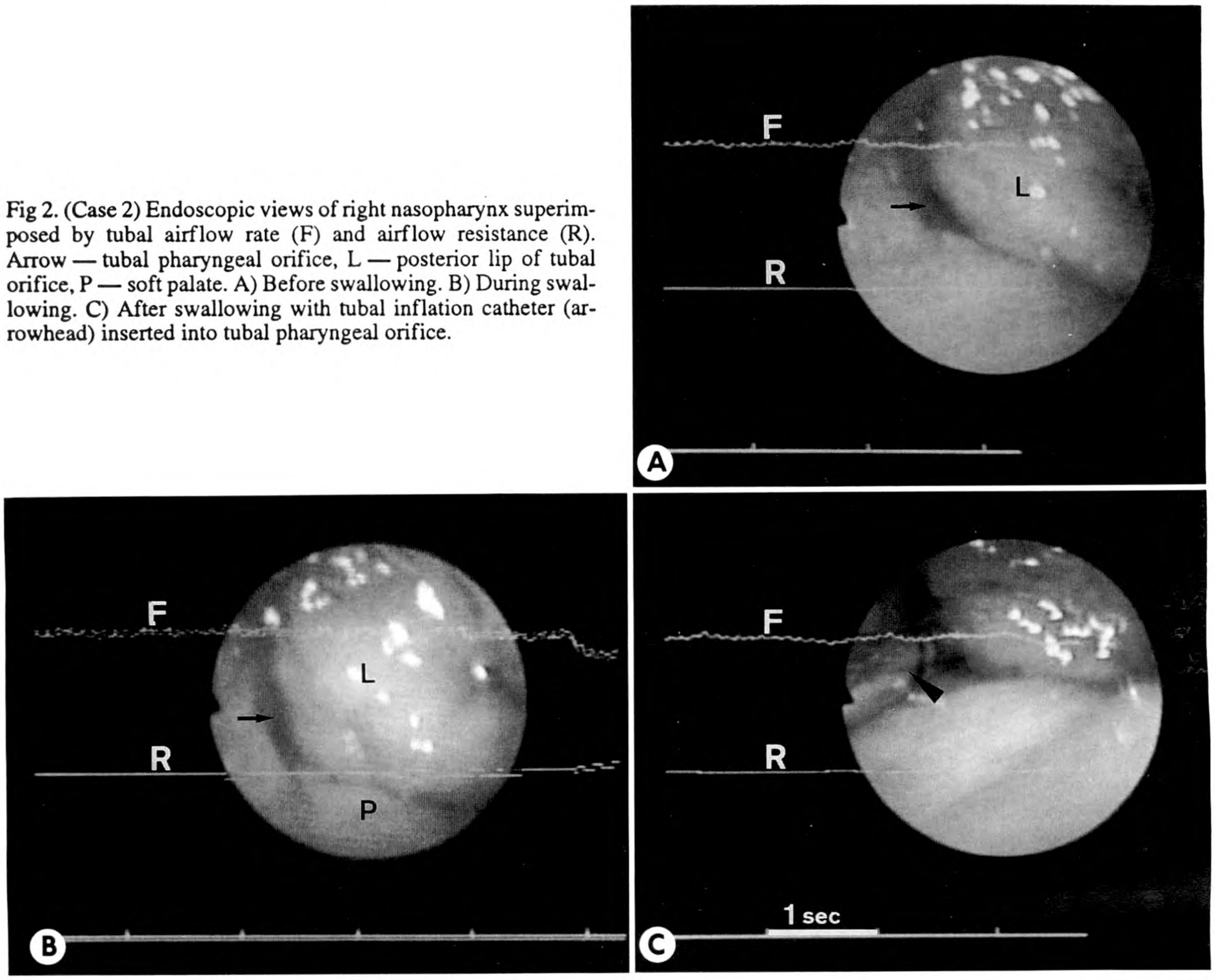

Fig 2. (Case 2) Endoscopic views of right nasopharynx superimposed by tubal airflow rate (F) and airflow resistance (R). Arrow — tubal pharyngeal orifice, L — posterior lip of tubal orifice, P — soft palate. A) Before swallowing. B) During swallowing. C) After swallowing with tubal inflation catheter (arrowhead) inserted into tubal pharyngeal orifice.

swallowing, despite obvious constriction of the ET as seen on the forced response test.

Among 7 ears of adults, edema (5 ears, 71%) was the most frequent cause, followed by unknown causes in 2 ears (29%); 1 did not show constriction on endoscopy, despite a clear constriction on the forced response test, and the other showed constriction on the forced response test after elevation of the soft palate, with no other remarkable finding observed on endoscopy.

DISCUSSION

The present study clearly demonstrated that the constriction of the ET during swallowing was due to squeezing of the tubal orifice by the elevated soft palate and various inflammatory conditions in the nasopharynx, indicating that the constriction occurred around the pharyngeal end of the ET. This was confirmed by the fact that insertion of an inflation catheter into the pharyngeal orifice of the ET prevented tubal constriction in an adult patient with OME (case 2). This agrees well with our previous finding that the site of tubal dysfunction lies near the pharyngeal orifice in most patients with OME.[7] The present observations also showed that the tubal constriction occurred together with the elevation of the soft palate in most of the patients examined. On swallowing, contraction of the levator veli palatini muscle (LVPM), causing the elevation of the soft palate, usually occurs before contraction of the tensor veli palatini muscle (TVPM), the dilator of the ET, and the time lag has been reported to be approximately 0.2 second in cats.[8] The ETs of OME patients, therefore, are not opened by the contraction of the TVPM, because the pharyngeal orifice has already been constricted by the contraction of the LVPM.

It should also be noted that the causes of this abnormal behavior of the ET were clearly related to inflammatory conditions in the nasopharynx such as hypertrophic adenoid, edema, and nasal discharge. When the present results are compared with our previous pathologic endoscopic findings of the ETs of OME patients,[9] the incidence of nasal discharge appears to be smaller in the present study. This might be because nasal discharge was sometimes cleaned up before endoscopy for clear visualization of the

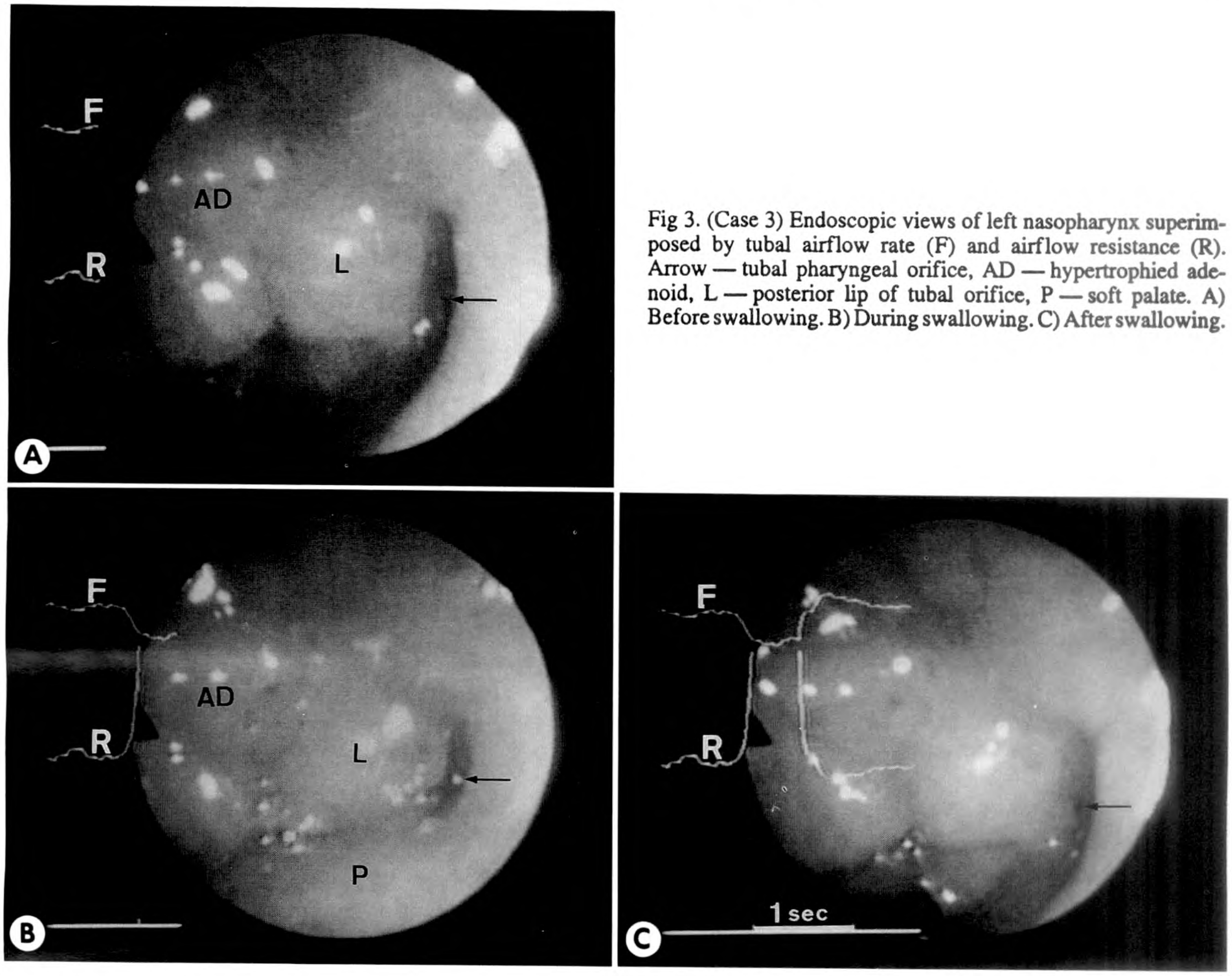

Fig 3. (Case 3) Endoscopic views of left nasopharynx superimposed by tubal airflow rate (F) and airflow resistance (R). Arrow — tubal pharyngeal orifice, AD — hypertrophied adenoid, L — posterior lip of tubal orifice, P — soft palate. A) Before swallowing. B) During swallowing. C) After swallowing.

nasopharynx and tubal orifice in this study. Nasal discharge, therefore, might be more frequent as a cause of tubal constriction in OME patients.

Although the sample size was small, we previously found that the active resistance of the ET during swallowing did not show significant improvement after adenoidectomy.[10] Children in whom tubal constriction appeared to be due to adenoid hypertrophy might also have other pathologic factors, such as

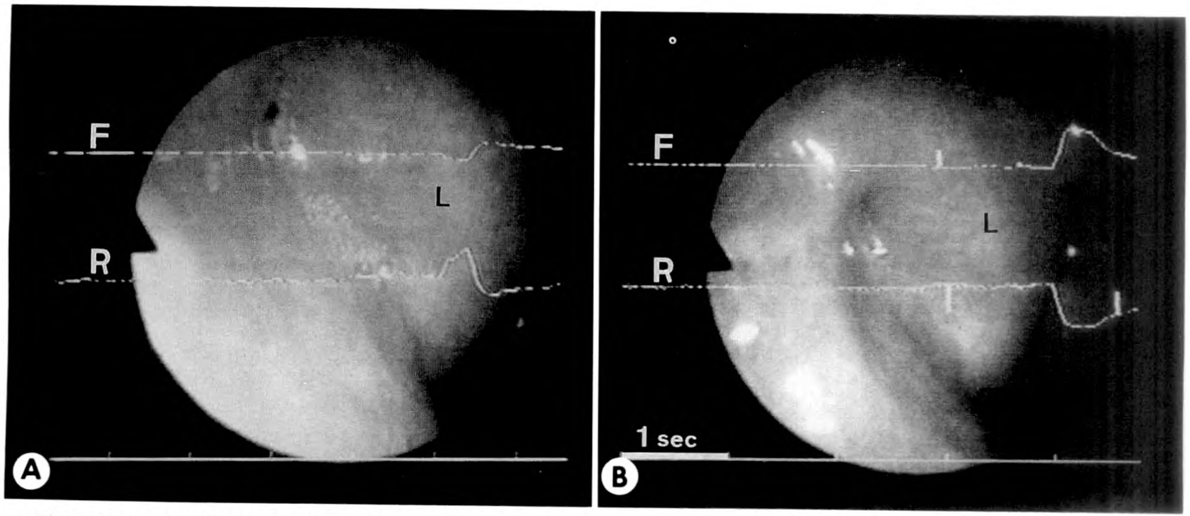

Fig 4. (Case 4) Endoscopic views of right nasopharynx superimposed by tubal airflow rate (F) and airflow resistance (R). L — posterior lip of tubal orifice. A) Before treatment of sinusitis. B) After treatment.

nasal discharge or edema around the pharyngeal orifice, that are hidden by the hypertrophied adenoid. Endoscopic follow-up study of adenoidectomized children is required to solve this question.

In an ear of an adult OME patient (case 4), the ET at first constricted during swallowing, but came to dilate as the edema of the posterior lip decreased after treatment of her paranasal sinusitis. This supported our sense that treatment of inflammatory conditions in the nasopharynx is important for improving tubal function.

REFERENCES

1. Silverstein H, Miller GF, Lindeman RC. Eustachian tube dysfunction as a cause of chronic secretory otitis in children. Laryngoscope 1966;76:259-73.
2. Bluestone CD, Klein JO. Otitis media in infants and children. Philadelphia, Pa: WB Saunders, 1988:15-30.
3. Honjo I. Eustachian tube and middle ear diseases. Tokyo, Japan: Springer-Verlag, 1988:39-74.
4. Cantekin EI, Saez CA, Bluestone CD, Bern SA. Airflow through the eustachian tube. Ann Otol Rhinol Laryngol 1979; 88:603-12.
5. Lildholdt T, Cantekin EI, Bluestone CD, Rockette HE. Effect of topical decongestant on eustachian tube function in children with tympanostomy tube. Acta Otolaryngol (Stockh) 1982;94:93-7.
6. Takahashi H, Hayashi M, Honjo I. Assessment of eustachian tube function by measuring tubal resistance. J Otolaryngol Jpn 1986;89:1825-30.
7. Takahashi H, Fujita A, Honjo I. Site of eustachian tube dysfunction in patients with otitis media with effusion. Am J Otolaryngol 1987;8:361-3.
8. Honjo I, Okazaki N, Kumazawa T. Experimental study of the eustachian tube function with regard to its related muscles. Acta Otolaryngol (Stockh) 1979;87:84-9.
9. Takahashi H, Honjo I, Fujita A. Endoscopic findings of pharyngeal orifice of the eustachian tube in otitis media with effusion. Eur Arch Otorhinolaryngol (in press).
10. Takahashi H, Fujita A, Honjo I. Effect of adenoidectomy on otitis media with effusion, tubal function and sinusitis. Am J Otolaryngol 1989;10:208-13.

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SPEECH RESULTS AND COMPLICATIONS OF NEAR-TOTAL LARYNGECTOMY

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Near-total laryngectomy provides a functional alternative to total laryngectomy in selected cases of laryngeal and hypopharyngeal cancer. We report our experience with the first 49 patients treated with near-total laryngectomy over a 3-year period. Successful speech rehabilitation was obtained in 79.5% of the patients in a median time of 40 days. Speech was acquired in 89.6% of the patients with follow-up longer than 6 months. Hands-free conversation was possible in 51.4% of the speaking patients. Five patients had symptomatic aspiration and 23 developed postoperative pharyngocutaneous fistula. Four local recurrences occurred in the laryngeal remnant. These results confirm that near-total laryngectomy provides a relatively safe, simple, and reproducible speech rehabilitation method for patients in whom total laryngectomy otherwise would be required.

KEY WORDS — laryngeal cancer, near-total laryngectomy, voice preservation.

INTRODUCTION

The key to complete success in oncologic surgery is radicality against cancer, not against the organ. Entire tumor removal does not necessarily require total organ resection. This is especially true with pharyngolaryngeal cancer, in which functional surgery plays an important role. Vertical partial laryngectomy and supraglottic laryngectomy are good examples of radical cancer treatment with function preservation.

The gap between the classic functional procedures and conventional total laryngectomy has been occupied by several "phonatory" techniques in which every effort is made to preserve speech while removing the larynx.[1-3] Most of these operations have been abandoned because of their morbidity and complications, mainly manifested by aspiration. This led to the development of prosthetic devices connecting the gullet to the lower airway.[4,5] However, tracheoesophageal (TE) puncture techniques are not a panacea. They demand periodic maintenance and frequent changing of the valve. Also, prostheses have the possibility of accidental extrusion with subsequent TE puncture closure.

The near-total laryngectomy was developed by Pearson et al[6] in 1980. The procedure was initially called extended hemilaryngectomy, then subtotal laryngectomy,[7] and finally near-total laryngectomy. From a surgical point of view, the operation resembles more a total than a partial laryngectomy, since the entire larynx is resected except for a narrow posterolateral strip connecting the trachea to the pharynx. The recurrent laryngeal nerve is preserved and a dynamic shunt is created with this strip, including the arytenoid and the posterior part of the vocal cord on the less involved side.

This report describes our experience with near-total laryngectomy over a 3-year period, emphasizing the functional results and complications of the operation. Although oncologic results will also be mentioned, no final conclusions can be drawn, since the follow-up is too short.

MATERIAL AND METHODS

From October 1, 1991, to September 30, 1994, 49 patients underwent near-total laryngectomy at the Departments of Otorhinolaryngology of La Paz (37 patients) and Juan Canalejo (12 patients) hospitals. All patients were male, with a mean age of 61 years (range 36 to 78).

One patient underwent surgery because of severe aspiration. The remaining 48 had diagnoses of squamous cell carcinoma (Table 1). None of these tumors were previously irradiated. Postoperative radiotherapy was used in 7 patients (14.3%). A total dose of 6,500 cGy was delivered in a schedule of 200 cGy per day, 5 days a week. Indications for postoperative radiotherapy included close resection margins, positive margins, and multiple nodal disease. The near-total operation was performed as a salvage procedure in 1 patient with a local recurrence 6 months after cordectomy. Table 2 documents the clinical stage of the

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From the Department of Otorhinolaryngology, La Paz Hospital, Autonomous University, Madrid (Gavilán, Prim, Rabanal), and the Department of Otorhinolaryngology, Juan Canalejo Hospital, La Coruña (Herranz), Spain.

TABLE 1. PATIENTS UNDERGOING NEAR-TOTAL LARYNGECTOMY BY PRIMARY SITE

Site	*n*
Supraglottic	23
Glottic	11
Transglottic	5
Hypopharynx	8
Tongue base	1

Total is 49 patients; 1 patient was treated because of permanent aspiration.

TABLE 2. STAGE OF PRIMARY TUMOR

	N0		*N1*		*N2*		*N3*	
	No.	*%*	*No.*	*%*	*No.*	*%*	*No.*	*%*
T1	2	4.2	0	0	0	0	0	0
T2	10	20.8	1	2.1	3	6.3	0	0
T3	14	29.2	2	4.2	6	12.5	1	2.1
T4	5	10.4	2	4.2	1	2.1	1	2.1

primary tumor and the neck for oncologic patients. In cancer patients, near-total laryngectomy was indicated either for oncologic (tumor-dependent) or physiologic (patient-dependent) reasons. The most frequent physiologic indication was poor cardiopulmonary function in elderly patients not suited to standard supraglottic laryngectomy.

Near-total laryngectomy was performed according to the method described by Pearson and DeSanto,[8] with only two significant variations. First, the larynx is approached through the vallecula — as for supraglottic laryngectomy — in all patients in whom this area is not invaded by the tumor (the majority of our cases). The second difference is that the shunt is created by tubing the mucosa around a 12F, instead of a 14F, calibrating catheter.

Extended procedures were required for 11 patients. Nine of them underwent a near-total pharyngolaryngectomy. Reconstruction was used in 6 of these cases, either with the pectoralis major flap or with the apron platysma myocutaneous flap.[9] Subtotal or total glossectomy was necessary in 2 patients. A total of 40 patients underwent some type of neck dissection as part of the primary treatment: 12 patients underwent unilateral functional neck dissection (FND), 20 patients underwent bilateral simultaneous FND, 5 patients underwent FND on one side of the neck and radical neck dissection (RND) on the other side, 2 patients underwent unilateral RND, and 1 patient underwent a two-stage bilateral RND. Therapeutic neck dissection was performed in patients with palpable nodes. High-risk patients with nonpalpable nodes — mainly supraglottic and hypopharyngeal tumors — underwent elective neck dissection. Sixteen patients had histologically positive nodes.

Follow-up ranged from 2 to 28 months, with a mean of 12 months. One patient with speech was lost to follow-up 18 months after the operation with no evidence of disease. During follow-up 3 patients developed second primary tumors (2 pharyngeal and 1 urologic). Speech results and complications have been evaluated from a clinical point of view. Statistical analysis was performed by the χ^2 test with Yates' continuity correction and the Mann-Whitney U test.

RESULTS

Five patients died in the postoperative period before they were evaluable for speech results and shunt complications. Two of them died because of cardiopulmonary complications, 2 suffered cerebral vascular attacks, and 1 died of pancreatitis. Successful lung-powered fistula speech for normal communication purposes according to the patient's opinion was acquired in 79.6% of the cases (35/44). The median time between surgery and speech acquisition was 40 days (range 11 to 180, mean 56). Among 29 patients with follow-up longer than 180 days, 26 (89.7%) were successfully rehabilitated. Although no relationship could be demonstrated between age and speech acquisition, the 3 patients without speech 6 months after the operation were older than the general population of the study (72.7 versus 61.3 years). Eighteen of the 35 speaking patients (51.4%) use the Barton button[10] in combination with the Blom-Singer tracheostoma valve for hands-free speech.

Five of the 44 evaluable patients (11.4%) had symptomatic grade III aspiration requiring treatment. The shunt was taken down in 1 patient — the first case in this series — and 2 patients underwent successful revision procedures. The remaining 2 patients are waiting for a surgical narrowing of the shunt. Five patients (11.4%) with grade I aspiration, ie, tracheal stain after swallowing, did not require further treatment. Symptomatic aspiration developed secondary to a local recurrence in another patient.

Pharyngocutaneous fistula was a major complication. Fifty percent of 46 evaluable patients developed a fistula. This complication was more frequent in patients undergoing surgery at La Paz Hospital (22/35) than in patients operated on at Juan Canalejo Hospital (1/11). In 4 cases the fistula required surgical closure. Salivary fistula significantly increased hospitalization. The median hospital stay was 22 days (range 10 to 75, mean 27). The average hospitalization was 37 days in patients with salivary fistula and 17 days in patients without fistula ($p < .001$).

Of the 48 oncologic patients, 5 (10.4%) developed local recurrences that were treated with radical sur-

TABLE 3. ANALYSIS OF RECURRENCES

Primary Site	*Stage*	*Neck Dissection*	*Postoperative XRT*	*Location of Recurrence**	*Time to Failure (mo)*	*Status*
Tongue base	T3N3M0	FND + RND	No	Local and distant	3	DOD
Glottic	T1N0M0	No	No	Local and neck	13	DOD
Supraglottic	T2N0M0	Unilateral RND	Yes	Distant	16	AWD
Supraglottic	T3N2cM0	Bilateral FND	No	Neck	13	ANED
Supraglottic	T2N2bM0	Bilateral FND	No	Neck	3	DOD
Supraglottic	T4N2cM0	FND + RND	No	Local	6	AWD
Supraglottic	T3N0M0	Unilateral FND	No	Local and neck	3	ANED
Supraglottic	T4N0M0	Bilateral FND	No	Local	4	ANED

Neck dissection and postoperative radiotherapy (XRT) were part of primary treatment.

FND — functional neck dissection, RND — radical neck dissection, DOD — dead of disease, AWD — alive with disease, ANED — alive with no evidence of disease.

*Only first failure site has been considered in text for evaluation of recurrences.

gery and radiotherapy. Two (4.2%) regional recurrences and 1 (2.1%) distant metastasis developed in patients with controlled primary tumor. Radiotherapy was used in 1 regional recurrence. The other regional recurrence and the patient with distant metastasis were not treated. The analysis of recurrences and their outcome is shown in Table 3. Thirty-six oncologic patients (75%) are alive without evidence of disease a mean of 12 months after the operation; 4 patients died because of the tumor; and the remaining patient is alive with disease. Seven patients (14.6%) died of causes unrelated to cancer, 5 of them during the postoperative period.

DISCUSSION

Despite the good oncologic and functional results obtained at the Mayo Clinic over the last 20 years,[11] near-total laryngectomy has not gained wide acceptance. It is well known that functional laryngeal surgery has never attracted much support in the United States, where radiotherapy for primary treatment and total laryngectomy for salvage have been the standards of treatment in many institutions. However, the functional usefulness of partial laryngectomy and its oncologic safety have stood the test of time.

While the speech results of near-total laryngectomy are comparable to those of TE puncture from a clinical standpoint, the near-total operation does not have the disadvantages of prosthetic rehabilitation. An important number of patients in our environment develop a permanent link with the hospital through the voice prosthesis, persisting even after they are cured of the disease. This results in a high rate of prosthesis withdrawal in the long term.[12] With the near-total operation this is not a problem, because the shunt does not need to be cleaned or replaced. Another advantage of the near-total laryngectomy over TE punctures is the high rate of patients who are able to speak without finger occlusion of the tracheostoma by using the Blom-Singer valve and the Barton button. More than 50% of the speaking patients in this series are regular hands-free speakers. This could be attributed to the physiologic speech mechanisms involved in near-total laryngectomy voice production. Since the patient's own innervated vocal cord is what produces the sound — as has been documented by means of video fiberoptic laryngoscopy — less pressure is needed and the tracheostoma valve is more easily adapted. In any case, TE puncture and near-total laryngectomy are not opposite procedures but rather, are complementary. The near-total operation offers an alternative to total laryngectomy in selected cases, while prosthetic rehabilitation still has its place in patients in whom total laryngectomy is the only surgical option available.

Aspiration was a main concern when near-total laryngectomy was incorporated into our surgical armamentarium. We prefer to have slow development of phonation and early swallowing than the opposite. For this reason we decided to use a 12F instead of a 14F calibrating catheter for shaping the shunt. However, this led to a similar aspiration rate when compared to Pearson's[13] initial results (11.2% versus 12.1% of near-total patients requiring revision procedures). Aspiration patients include an obvious technical failure in our first case, in whom a very large shunt was created. Symptomatic aspiration can be easily solved by means of surgical narrowing of the shunt. Patients developing late aspiration problems should be carefully examined for local recurrence.

The most discouraging aspect of our early experience with near-total laryngectomy has been the high rate of pharyngocutaneous fistula. Although it does not represent a major problem from an oncologic or functional standpoint, pharyngocutaneous fistula is a bothersome complication with social and economic impact. It delays hospital discharge and increases the costs of the operation. Although 22.4% of our patients underwent extended near-total laryngectomies, requiring major reconstructive procedures in some cases, an overall incidence of salivary fistula of 50% has never before been reported in relation to near-total laryngectomy. The problem is even more striking since almost all fistulae developed in the institution with the larger near-total experience. A technical failure of the hypopharyngeal suture was initially considered responsible for this complication. The surgical details were discussed with surgeons from other centers, but no positive conclusions were reached. Meanwhile, a different study performed in our institution concerning the usefulness of prophylactic anticoagulation with low–molecular weight heparin in major head and neck surgery revealed that our treatment protocol led to an increase in several complications without a significant improvement in pulmonary embolism or postoperative survival. The incidence of pharyngocutaneous fistula, hematoma, and wound dehiscence was significantly higher in patients with prophylactic anticoagulation than in patients not receiving anticoagulation. Therefore, the anticoagulation protocol was discontinued and the incidence of pharyngocutaneous fistula in patients undergoing near-total laryngectomy has substantially decreased since then. With the data available, we can only suspect that the anticoagulation protocol might have been a contributing factor for pharyngocutaneous fistula. More cases are needed to confirm a direct relationship between prophylactic anticoagulation and pharyngocutaneous fistula.

Our high postoperative fatality rate (10.2%) should be examined in light of the indications for near-total laryngectomy in this series. Nearly 50% of our patients (23/49) underwent the operation for physiologic reasons. These are patients whose tumors were suitable for supraglottic laryngectomy, but in whom, because of their poor general condition, conventional conservation procedures would have been dangerous or impossible. Therefore, the cases in this series represent a selection of patients with a bad prognosis from a surgical standpoint. In fact, only 2 unilateral FNDs were performed in the 5 patients who died in the postoperative period. Under normal conditions, 8 neck dissections should have been performed in these cases (1 patient had a glottic lesion), since bilateral FND is our standard for patients with supraglottic and hypopharyngeal tumors.

Five of the 8 recurrences developed in stage IV patients. Among local recurrences, 3 developed in stage IV patients, 1 in a stage III patient, and 1 in a stage I patient. The histopathologic report of the stage I patient dying of locoregional failure revealed lymphatic permeation without neck node metastasis. The short follow-up available prevented us from drawing oncologic conclusions. However, the oncologic safety of near-total laryngectomy has been sufficiently proved by histopathologic studies[14,15] and by the Mayo Clinic experience.[11]

CONCLUSION

Near-total laryngectomy is a useful option in our practice for the surgical treatment of patients who are candidates for conservation surgery but will not tolerate it for reasons of age or health. It is also appropriate for some selected unilateral cord-fixing tumors unsuitable for conventional conservation procedures. The near-total operation enlarges the field of conservation laryngeal surgery with a new concept of voice preservation. It provides an additional surgical option that should help us to avoid the temptation to stretch the indications for the classic conservation procedures. Near-total laryngectomy enlarges the speech rehabilitation options for the laryngectomized patient by adding a physiologic, nonprosthetic tissue technique.

REFERENCES

1. Staffieri M, Staffieri A. Rehabilitación quirúrgica de la voz mediante la técnica de la neoglotis fonatoria. In: Algaba J, ed. Recuperación de la voz en los laringuectomizados. Fistuloplastias y prótesis fonatorias. Madrid, Spain: Garsi, 1988:163-9.
2. Mozolewski ES, Zietec E, Wysocki R, Jach K, Jassem W. Arytenoid vocal shunt in laryngectomized patients. Laryngoscope 1975;85:853-61.
3. Arslan M, Serafini I. Restoration of laryngeal functions after total laryngectomy. Report on the first 25 cases. Laryngoscope 1972;82:1349-60.
4. Singer MI, Blom ED. An endoscopic technique for restoration of voice after laryngectomy. Ann Otol Rhinol Laryngol 1980;89:529-33.
5. Panje WR. Prosthetic vocal rehabilitation following laryngectomy. The voice button. Ann Otol Rhinol Laryngol 1981; 90:116-20.
6. Pearson BW, Woods RD, Hartman DE. Extended hemilaryngectomy for T3 glottic carcinoma with preservation of speech and swallowing. Laryngoscope 1980;90:1950-61.
7. Pearson BW. Subtotal laryngectomy. Laryngoscope 1981; 91:1904-12.
8. Pearson BW, DeSanto LW. Near-total laryngectomy. Operative Techniques Otolaryngol Head Neck Surg 1990;1:28-41.
9. Bernáldez R, Cerdeira MA, Gavilán J. Pharyngeal reconstruction with the apron platysma myocutaneous flap. Operative Techniques Otolaryngol Head Neck Surg 1993;4:303-5.
10. Barton D, DeSanto LW, Pearson BW, Keith R. An en-

dostomal tracheostomy tube for leakproof retention of the Blom-Singer stomal valve. Otolaryngol Head Neck Surg 1988;99:38-41.

11. Pearson BW, DeSanto LW, Olsen KD. The functional and oncologic results of near-total laryngectomy. Proceedings of the Third International Conference on Head and Neck Cancer, San Francisco, Calif, July 26-30, 1992.

12. Quer M, Burgués-Vila J, García-Crespillo P. Primary tracheoesophageal puncture vs esophageal speech. Arch Otolaryngol Head Neck Surg 1992;118:188-90.

13. Pearson BW. Near-total laryngectomy. In: Cummings CW, Fredrickson JM, eds. Otolaryngology head and neck surgery. St Louis, Mo: CV Mosby, 1986:2117-32.

14. Robbins KT, Michaels L. Feasibility of subtotal laryngectomy based on whole-organ examination. Arch Otolaryngol 1985;111:356-60.

15. Kirchner JA, Som ML. Clinical significance of fixed vocal cord. Laryngoscope 1971;81:1029-44.

PEDIATRIC OTORHINOLARYNGOLOGY ENDOSCOPY DAYS

The First International Days in Pediatric Otorhinolaryngology Endoscopy will be held in Lyon, France, October 14-15, 1996. For further information, contact Marie-Claude Duchez, Departement d'ORL et de Chirurgie Cervico-Faciale, Hôpital E. Herriot, Place d'Arsonval, 69003 Lyon, France; fax 33 72 11 05 34.

SUPRAESOPHAGEAL COMPLICATIONS OF REFLUX DISEASE

Supraesophageal Complications of Reflux Disease will be held November 21-23, 1996, in Tucson, Arizona. For further information, contact Reza Shaker, MD, MCW Dysphagia Institute, Division of Gastroenterology, Froedtert Memorial Lutheran Hospital, 9200 W Wisconsin Ave, Milwaukee, WI 53226; telephone (414) 259-2742; fax (414) 384-8480.

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RECORDINGS OF MUCOCILIARY ACTIVITY IN VIVO: BENEFIT OF FAST FOURIER TRANSFORMATION OF THE PHOTOELECTRIC SIGNAL

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Investigations of mucociliary activity in vivo are based on photoelectric recordings of light reflections from the mucosa. The alterations in light intensity produced by the beating cilia are picked up by a photodetector and converted to photoelectric signals. The optimal processing of these signals is not known, but in vitro recordings have been reported to benefit from fast Fourier transformation (FFT) of the signal. The aim of the investigation was to study the effect of FFT for frequency analysis of photoelectric signals originating from an artificial light source simulating mucociliary activity or from sinus or nasal mucosa in vivo, as compared to a conventional method of calculating mucociliary wave frequency, in which each peak in the signal is interpreted as a beat (old method). In the experiments with the artificial light source, the FFT system was superior to the conventional method by a factor of 50 in detecting weak signals. By using FFT signal processing, frequency could be correctly calculated in experiments with a compound signal. In experiments in the rabbit maxillary sinus, the spontaneous variations were greater when signals were processed by FFT. The correlation between the two methods was excellent: $r = .92$. The increase in mucociliary activity in response to the ciliary stimulant methacholine at a dosage of 0.5 µg/kg was greater measured with the FFT than with the old method (55.3% ± 8.3% versus 43.0% ± 8.2%, $p < .05$, $N = 8$), and only with the FFT system could a significant effect of a threshold dose (0.05 µg/kg) of methacholine be detected. In the human nose, recordings from aluminum foil placed on the nasal dorsum and from the nasal septa mucosa displayed some similarities in the lower frequency spectrum (<5 Hz) attributable to artifacts. The predominant cause of these artifacts was the pulse beat, whereas in the frequency spectrum above 5 Hz, results differed for the two sources of reflected light, the mean frequency in seven healthy volunteers being 7.8 ± 1.6 Hz for the human nasal mucosa. It is concluded that the FFT system has greater sensitivity in detecting photoelectric signals derived from the mucociliary system, and that it is also a useful tool for analyzing the contributions of artifacts to the signal.

KEY WORDS — cilia, human, maxillary sinus, mucociliary clearance, nasal mucosa, rabbit.

INTRODUCTION

The mucociliary system is the first line in airway defense against noxious stimuli and agents from the environment. Inhaled particles, bacteria, and viruses are trapped in the mucus that covers the airways and are transported by the beat of the cilia to the pharynx, where they are either swallowed or coughed up. The mucous membranes in the nose, paranasal sinuses, and tubal part of the middle ear are all covered with a ciliated epithelium, and the same types of cells are found in the bronchi and trachea. Nasal cilia are approximately 6 µm in length, and every ciliated cell has 50 to 200 cilia beating continuously.[1] The locomotion of cilia is achieved through temporal and spatial coordination known as metachronism. Factors affecting the ciliary beat frequency (CBF) and the composition of airway secretions regulate the efficiency of the transport of mucus.

As the driving force of mucus transport is the beat of cilia, it is important to investigate the CBF. These measurements are usually done in vitro, where CBF can be measured with photometric techniques. The sample containing the beating cilia is observed through a microscope. The beam of light from the microscope passes through the specimen, and variations of the light intensity produced by the beating cilia are picked up by a sensitive photodetector. The best signals derive from cells viewed from the side; ie, their ciliated brush border is clearly seen. Variations in the electrical current produced by the photodetector are processed for calculations of the CBF. Apart from the variations in the photoelectric current that are caused by ciliary beat, the signal also contains an amount of background noise, due to movements in the environment or movements within the ciliated cells themselves. Noise is also generated in the photodetector and in the amplifiers. In an attempt to improve the system's capability of extracting the CBF from the photoelectric signal, the fast Fourier transformation (FFT) has been used in the signal processing.[2,3] The FFT analysis of the signal is here used to produce a diagram displaying the amplitudes of the various frequency components that exist in the photoelectric

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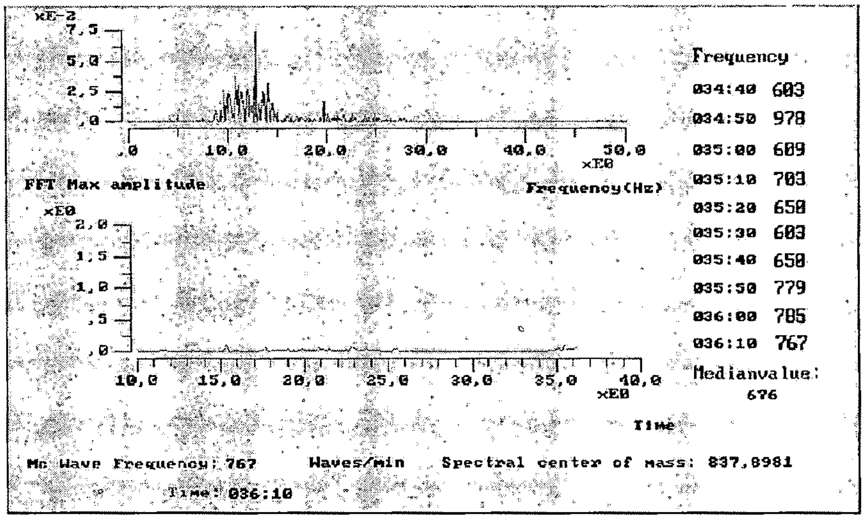

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Frequency
034:40  603
034:50  978
035:00  609
035:10  703
035:20  650
035:30  603
035:40  650
035:50  779
036:00  785
036:10  767
Medianvalue:
   676

Mc Wave Frequency: 767    Waves/min    Spectral center of mass: 837,8981
        Time: 036:10
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Fig 1. Fast Fourier transformation (FFT) of ciliary beat frequency in sample from human nasal mucosa. Illustration is screen dump from software Viewdac, used for analysis. Table to right of diagram shows latest 10 FFT calculations of ciliary beat frequency, and median of these.

signal. A typical example is shown in Fig 1. The predominant CBF is defined as the frequency with the highest amplitude in the diagram.

However, in vitro investigations of CBF have limitations. Although pharmacologic studies with inflammatory mediators and agonists and antagonists exerting effects on the autonomic nervous system enable hypotheses to be generated as to the regulation of the mucociliary system, the confirmation of such hypotheses requires in vivo models for physiological experiments. Few such models have been described. Experimental work has been done in the rabbit maxillary sinus by our own group[4] and in the canine trachea,[5] representing the upper and lower airways, respectively. Light passing through the preparation is used in in vitro measurements of CBF, whereas reflected light is used in in vivo recordings of mucociliary activity. The cilia beating beneath the mucus carpet produce a flickering light reflection if a light beam of any kind is directed at an angle against the mucosa. The variations in the light reflection are picked up by a photodetector, and transformed into electrical signals that are processed for calculations of the mucociliary wave frequency. It is generally assumed that the signal represents an averaged CBF from many adjacent cells. However, the effect of small frequency differences between adjacent cells is unknown, nor is it known whether the metachronal wave in the mucus produced by the beating cilia contributes to the photoelectric signal obtained in vivo.

The main drawback to using experimental animal models is the mandatory need for anesthesia, which may damp important airway reflexes known to regulate mucociliary function.[6] Furthermore, a direct effect of the anesthesia on CBF cannot be excluded. It has also been shown that surgical trauma will affect CBF.[7] For these reasons, we developed an in vivo model for recordings of mucociliary activity in the human nose that facilitates measurements without the use of anesthesia of any kind.[8] However, the problem with artifacts caused by movements, for example respiration or pulse beat, is greater in vivo than in vitro. Moreover, the reflected light from the human nasal mucosa is weaker than in animal models, as very fine optical fibers are used both for incoming light and for the reflected signal. It has been reported that in vitro recording of CBF has benefited from FFT of the signal. Whether the use of FFT can improve the experimental system's capability of correctly calculating the mucociliary wave frequency also in vivo is not known. The aim of the present investigation was therefore to investigate the effect of using FFT for frequency analysis on photoelectric recordings of mucociliary activity in vivo and on signals produced by an artificial light source simulating mucociliary activity, and to compare this method for processing photoelectric signals with a conventional peak-counting method for calculating mucociliary activity.

METHODS

The photoelectric signal in the present investigation was current-to-voltage converted, filtered, and amplified by a custom-made amplifier built by the Biomedical Engineering Department, University Hospital, Lund, Sweden. The signal was amplified 500 or 1,000 times and, after passing the amplifier, displayed on an oscilloscope. The output from the amplifier was picked up by two different systems for signal processing.

Conventional System. Recordings of mucociliary activity were analyzed by an IBM 30 AT computer (80286 processor running at 10 MHz) equipped with a 12-bit analog to digital converter (ADC) board (CA0300, CE-Bit Elektronik AB, Solna, Sweden).

The photoelectric signals were processed by a custom-made computer program with an algorithm counting each change of polarity of the derivative of the signal (eg, a maximum or minimum) as a new mucociliary wave, provided that there had been a sufficient change in amplitude since the last count, thus reducing the risk of counting noise.[9] The mucociliary wave frequency was calculated each 10 seconds. This equipment, or an almost identical prototype, has been in use in the laboratory since 1981 and conforms to the tradition of manually counting the mucociliary wave frequency on strip charts or plotter printouts. This method is hereinafter referred to as "old."

Fast Fourier Transformation System. Fast Fourier transformation of identical raw signals from the amplifier was performed by a Hewlett-Packard Vectra (80486 processor running at 66 MHz) equipped with a 12-bit ADC board (DAS-1401; Keithley, Taunton, Mass) and processed by the computer program Viewdac (version 2.1) from the same manufacturer. The sampling frequency was 100 Hz, and FFT was calculated on 1,024 data points each 10 seconds. Twenty-four zeros were appended to the 1,000 acquired data points. A rectangular window function was used. The Viewdac software permitted the investigator to select a window for the display of the FFT diagram, and this interval was set to 0.5 to 45 Hz in the experiments. This method is hereinafter referred to as FFT. The calculation of FFT is displayed as an x-y diagram with the abscissa showing the frequency and the ordinate displaying the amplitude of the power at the corresponding frequency. The FFT algorithm considered the frequency component of the spectrum that had the greatest power to be the mucociliary wave frequency in the investigated mucosa. All experiments were recorded on both computer systems simultaneously, if not otherwise stated.

Function Generator Experiments. A function generator (Wavetek Function Generator model 95, Wavetek, San Diego, Calif) was connected to a standard 5-mm red light–emitting diode (LED), in series with a current-limiting resistor. The output from the function generator was a sinusoidal voltage. A DC offset component was added to the sine wave in order to avoid extinction of the light from the LED during negative parts of the sine wave. The LED was positioned 50 mm from the diaphragm of the phototransistor used. The diaphragm has a diameter of 200 μm. The experiments were run in the laboratory in ordinary room background light and with the gain of the amplifier set to ×1,000. The threshold levels (expressed in millivolts) were determined for the following frequencies: 5, 7.5, 10, 15, 20, 30, and 40 Hz. The threshold voltage was defined as the lowest peak-to-peak voltage supplied to the LED and resistor at which the signal processing system was able to correctly calculate the CBF (input frequency as displayed on the function generator ± 10%) in at least 4 of 7 consecutive measurements. The threshold levels were plotted on a log scale as a function of the frequency. At the end of the experiments the background electrical noise in the system was recorded by turning the LED off and performing FFTs both in room lighting and in complete darkness.

In another series of function generator experiments, a compound signal was used for the experiments. The purpose of this experiment was to investigate the FFT system's ability to calculate the correct frequencies of simultaneous signals of different frequencies, simulating signal and noise. Two experimental series with three combinations were used (the numbers within parentheses refer to the relative intensity of the signal): *Series 1* I: 1 Hz (3), 4 Hz (3), and 7 Hz (1); II: 1 Hz (3), 4 Hz (3), and 10 Hz (1); III: 1 Hz (3), 4 Hz (3), and 15 Hz (1). *Series 2* I: 1.01 Hz (3), 4.05 Hz (3), and 7.08 Hz (1); II: 1.01 Hz (3), 4.05 Hz (3), and 10.12 Hz (1); III: 1.01 Hz (3), 4.05 Hz (3), and 15.18 Hz (1). The second series was done to investigate the effect on the FFT analysis of a small shift in frequency. All signals were sinusoidal and the voltage delivered to the LED varied between 4 and 40 mV. Only the FFT system was used in these experiments.

Animal Experiments. Adult white New Zealand rabbits weighing 2.1 to 3.5 kg were used in this study. The rabbits were housed in an approved animal facility, and the protocol was approved by the Committee for Animal Care and Use of the University of Lund. The photoelectric technique and its surgery has been reported previously.[4] In brief, under anesthesia with urethane (2 g/kg), a size 3F cannula (Portex Ltd, Hythe, England) was placed in the facial or lingual artery and continuously perfused with physiological saline at 2 to 5 mL/h to keep the inlet open. This intra-arterial route was used for administering methacholine, a known accelerator of mucociliary activity.[10] The mucosa was exposed via a trepanation in the ipsilateral maxillary sinus of about 2 × 8 mm. To prevent desiccation of the mucosa, the opening was covered with a window heated to approximately 38°C and sealed to the bone with bone wax (Ethicon, England). A light beam from a cold light source was directed on the maxillary sinus mucosa, producing a reflection from the mucus carpet. Mucociliary activity (visible as flickering light reflections) was observed through a binocular microscope (Zeiss, Oberkochen, Germany). Once distinct reflected light patterns had been identified, one eyepiece was replaced with a photodetector and the light fluctuations were recorded. The recordings were analyzed simultaneously with the two systems for signal processing, and the mucociliary wave frequency was calculated every 10 seconds. Induced frequency changes were expressed as percentages of the frequency zero level (the basal mucociliary wave frequency immediately preceding the administration of methacholine). Rectal temperature was monitored and body temperature was maintained at 37.0°C to 38.5°C with a heating pad. Methacholine (Sigma, St Louis, Mo) was dissolved in physiological saline. Bolus doses were administered in 0.2-mL volumes followed by a 0.3-mL flush of normal saline (the volume of tubing between the syringe and the cannula opening).

The experimental procedures were as follows:

1. The normal variations of mucociliary activity were determined in eight rabbits after bolus injections of physiological saline. Physiological saline is commonly used as a vehicle for various pharmacologic challenges.

2. Eight rabbits were injected intra-arterially with bolus dosages of 0.5 μg/kg methacholine, a dose that produces an increase of mucociliary activity of about 30%[10] and that is used as a reference challenge in the laboratory to determine whether a preparation is working properly.

3. The effect of 0.01 μg/kg and 0.05 μg/kg methacholine on mucociliary activity was recorded in seven rabbits. These doses are close to the theoretic threshold dose for methacholine in this animal model of 0.03 μg/kg.[10]

4. The morphometric characteristics of the recordings of mucociliary activity have previously been found to be important for the experiments, and the presence of a so-called herringbone pattern with alternating small and large amplitudes of the waves was used as an indicator that the photoelectrical signal was adequate and free of artifacts.[4] In seven rabbits a spot in the mucosa was selected that did not manifest this pattern, and was therefore considered an unsuitable signal source for mucociliary experiments. These rabbits were then challenged with 0.5 μg/kg methacholine.

Human Experiments. Seven male volunteers, 31 to 44 years of age, were enrolled in the study. The study design was approved by the Ethics Committee of the University of Lund. All subjects were nonsmokers, and none had a history of chronic sinusitis, nasal surgery, or systemic medication, or had had acute upper airway disease or nasal medication during the 3 weeks preceding the study.

The subject sat relaxed astride an upright chair, facing the back of the chair, which gave support to the chest, being instructed to lean slightly forward with the elbows resting on a corneal microscope table modified for this purpose. The lower jaw and the forehead were supported by a frame affixed to the corneal microscope stand, and the upper jaw was fixed by a plastic cast of the subject's upper teeth, screwed to the stand. Without local anesthesia, mucociliary activity was recorded from the nasal septum approximately 45 to 50 mm from the nasal vestibule at the level of the middle meatus, the probe being carefully advanced into the nose under direct visual control with a 25° nasoscope (Panoview Plus, Richard Wolf Gmbh, Knittlingen, Germany). The probe could be rotated along its axis and its position adjusted by means of micrometer screws. Light to and from the mucosa was transmitted by optical fibers with an inside diameter of 100 μm. Visual control of the light reflections was obtained by a separate optical fiber bundle.[8]

The mucociliary wave pattern was monitored continuously on an oscilloscope, and processed by the FFT signal processing system. The recordings of mucociliary activity were compared to subsequent recordings of light reflections produced by aluminum foil positioned on the nasal dorsum. The photoelectric signals from the septum and the aluminum foil were followed for 5 minutes each. This experiment was done in order to analyze the role of artifacts in the photoelectric signals produced by pulse, breathing, and body tremor, which are assumed to be approximately equally represented in the mucociliary activity and the aluminum foil recordings, whereas the signal derived from the ciliary beats is absent in the aluminum foil recording.

Statistics and Data Analysis. The results of each experiment was presented as the mean ± SEM. The correlation coefficients were calculated according to Pearson. The statistical test for the mucociliary experiments was Student's *t*-test for paired data (responses being normally distributed), with the exception of the experiments with poor light reflections in rabbits and in the human nasal mucosa, in which Wilcoxon's matched pairs signed-rank test (paired data) and the Mann-Whitney U-test (unpaired data) were used instead, owing to the non-Gaussian distribution of the responses. The induced frequency changes were expressed as percentages of the frequency zero level, ie, the basal mucociliary activity immediately before a challenge. Peak response and area under the curve (AUC) during a 5-minute observation period after a challenge were used for the evaluations. P values of < .05 were considered sig-

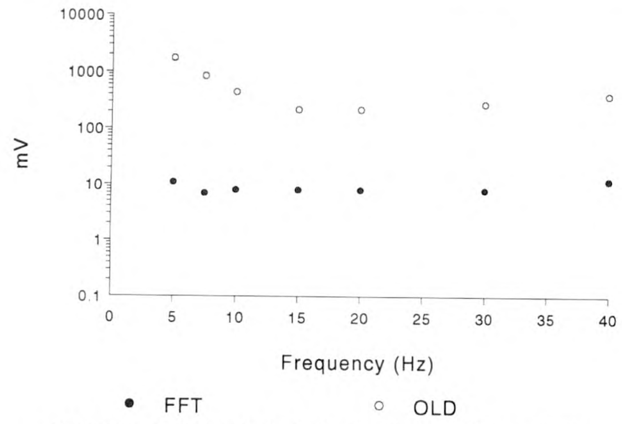

Fig 2. Threshold levels for both signal processing systems for detection of correct (tolerance ± 10%) frequency from standard 5-mm light-emitting diode as function of frequency. Threshold levels are plotted on log scale.

nificant.

RESULTS

Function Generator Experiments. The threshold levels were measured as peak-to-peak voltage delivered to the LED from the Wavetek function generator, at which the signal processing systems were able to correctly calculate the input frequency to the LED. The threshold levels differed considerably between the two processing systems for detection of mucociliary activity. The output voltage of the function generator needed to detect a signal correctly was much higher in the old processing system than in the FFT system, the respective threshold levels for adequate detection being approximately 0.5 V (range, 0.22 to 1.75 V) and 10 mV (range, 7 to 12 mV). Dividing 0.5 V by 10 mV gives a sensitivity difference factor between the two signal processing systems of 50 in favor of the FFT system. The comparison is even more favorable to the FFT system if one takes into account that the FFT system yielded the exact frequency in 98% of the readings, as compared to the 10% deviation from the true frequency allowed in the experiments with the old system. Had the same level of accuracy been required of the old system, the difference in the detection thresholds would have been even greater. Besides being the more sensitive of the two signal processing systems, the FFT system was more stable and the threshold level varied little between the various frequencies tested when the FFT system was investigated. In contrast, the threshold levels for the old signal processing system were frequency-dependent, and the sensitivity fell for frequencies below 15 Hz (Fig 2).

The noise in the system was found to be identical with or without the presence of background light. The power spectrum was in the 10^{-5} units (actually volts squared) range for noise, as compared to 10^{0} to 10^{-3} units for typical analysis of mucociliary activity or light fluctuations emitted by the LED (data not shown).

In another series of function generator experiments, a compound signal was used for the experiments and the signal was processed by FFT only. The system was found to correctly calculate the frequencies of the three components of the compound signal. Also, the relative amplitudes were correctly calculated, with the exception of the 1-Hz component, which was attenuated (Fig 3). The FFT amplitudes of the 7-, 10-, and 15-Hz components were approximately 10 times weaker than that of the 4-Hz component. The amplitudes of all components were dependent on the voltage used in a linear relation in the log-log plot. The small shift of the frequencies between series 1 and 2 did not influence the relative amplitudes when the different components were compared — only the absolute values of the amplitudes.

Animal Experiments. Bolus injections of physiological saline did not affect mucociliary activity. The maximum increase did not differ from the varia-

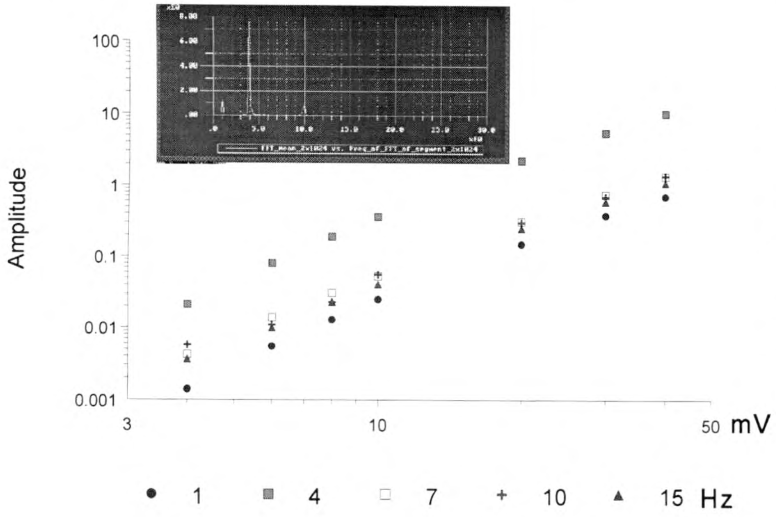

Fig 3. Results of combination experiments with function generator (which acted as "ciliary simulator"). Amplitudes in power spectrum at various inputs in log-log diagram. Note that amplitudes of frequencies in range 7 to 15 Hz overlap each other. Rectangular box displays typical power spectrum showing results of experiment with compound signal of 1.01, 4.05, and 10.12 Hz. Slopes of plots varied from 2.47 to 2.67, which is slightly higher than expected. Theoretic equation is $P_{FFT} = K \times U^2$, where P_{FFT} is amplitude in FFT analyses, K is constant, and U is voltage of current delivered to light-emitting diode; thus, slope of 2 is expected.

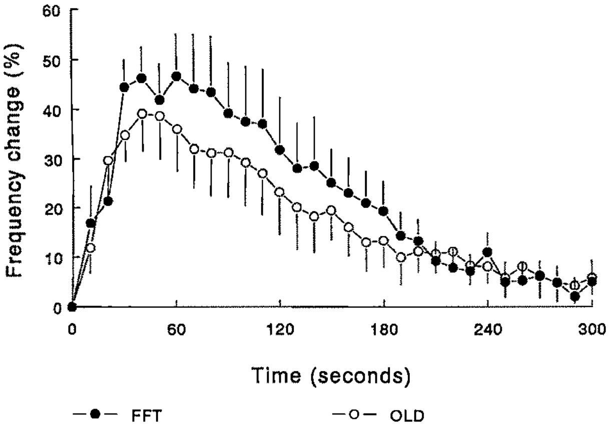

Fig 4. Time course of mucociliary activity in rabbit maxillary sinus of bolus dose of 0.5 μg/kg methacholine injected intra-arterially (N = 8).

tions measured before the bolus injections. However, the peak values during the 5 minutes after injection with saline were significantly higher when measured with FFT, 11.6% ± 1.7%, as compared to 6.6% ± 0.6% measured with the old system ($p < .05$, N = 8). The AUC for the saline experiments did not differ between the two systems for signal processing, the calculated areas being −17.8 ± 48.6 percentage seconds (FFT) and 22.3 ± 18.8 percentage seconds (old). The FFT analysis yielded greater spontaneous changes in the mucociliary wave frequencies than did the old system, manifested not only as a higher peak but also as a higher coefficient of variation in the saline experiments, the coefficients of variation being 5.9% ± 0.5% in the FFT analysis and 2.9% ± 0.2% in the old analysis ($p < .001$). A frequency zero level of 1,260 ± 242 waves per minute was obtained with the FFT system, and of 1,317 ± 64 waves per minute with the old system. This difference was not significant. Correlation of raw data obtained with the two methods of determining the frequency zero level showed correlation to be excellent (r = .92).

The increase in mucociliary activity after challenge with 0.5 μg/kg methacholine produced a prompt, transient increase of mucociliary activity, as expected. This increase was significantly higher with the FFT system than with the old system: 55.3% ± 8.3% versus 43.0% ± 8.2% ($p < .05$, N = 8). The AUC was also significantly larger in the FFT analysis, the FFT AUC being 685.33 ± 162.8 percentage seconds and the old AUC being 556.4 ± 169.0 percentage seconds ($p < .05$). When the peak responses were correlated, the slope was 0.84 and the coefficient of correlation r = .86, indicating a strong correlation between the two methods. Correlating the AUC showed an even better correlation between the two methods for signal processing; the slope was 1.0 and the correlation coefficient r = .96, indicating an almost ideal regression between the two methods. In contrast to the experiments with physiological saline, the frequency zero levels differed significantly between the two methods: 1,109 ± 64 waves per minute (FFT) versus 1,215 ± 50 waves per minute (old) ($p < .01$). Analysis of the temporary responses showed that FFT analysis yielded stronger increases of mucociliary activity than did the old system (Fig 4). This difference was consistent throughout the duration of the main response, ie, between 30 and 180 seconds after challenge.

Neither FFT nor the old system of analysis showed any effect of 0.01 μg/kg methacholine, as compared to saline. For the higher dose tested, 0.05 μg/kg methacholine, the FFT system revealed a significant change in the peak response, but the old system still detected no change in the mucociliary activity as compared to saline (Table 1).

Both methods of calculating mucociliary wave frequency showed a strong response after challenge with 0.5 μg/kg methacholine, even when the morphometric characteristics of the photoelectric signal were

TABLE 1. EFFECT OF METHACHOLINE AT DOSAGES OF 0.01 OR 0.05 MICROGRAMS PER KILOGRAM, AS COMPARED TO VEHICLE PHYSIOLOGICAL SALINE, ON MUCOCILIARY ACTIVITY IN RABBIT MAXILLARY SINUS

Challenge	*FFT*	*Old*	*pFFT*	*pOld*	*pMethods*
Peak responses					
NaCl	9.3% ± 2.0%	9.6% ± 1.1%			
0.01 μg/kg	16.6% ± 8.0%	11.8% ± 4.0%	NS	NS	NS
0.05 μg/kg	40.3% ± 14.8%	20.3% ± 4.8%	<.05	NS	<.05
AUC					
NaCl	−94.1%s ± 25.2%s	31.3%s ± 31.1%s			
0.01 μg/kg	−25.9%s ± 44.7%s	20.6%s ± 37.3%s	NS	NS	NS
0.05 μg/kg	205.6%s ± 191.8%s	117.4%s ± 52.1%s	NS	NS	NS

Values are means and SEM. Peak responses are percentage changes from frequency zero level immediately before challenge, and area under the curve (AUC) during 5 minutes after challenge is expressed as percentage seconds (%s) calculated with polygonal method. pFFT and pOld refer to comparisons with saline, and pMethods refers to comparisons between old (see text) and fast Fourier transformation (FFT) systems of signal processing.

NS — not significant.

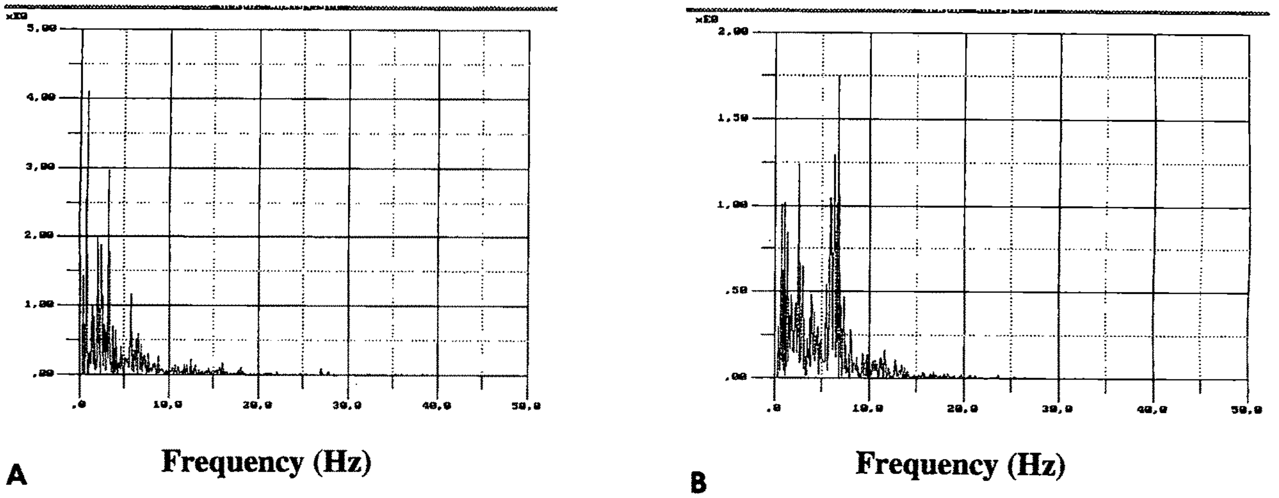

Fig 5. Example of FFT analysis of raw data in subject 3 (36-year-old man). Graphs are based on screen dumps from software Viewdac. A) Results of experiments with aluminum foil. B) Results obtained from nasal mucosa.

considered poor by the investigator. The mucociliary increase was 85.2% ± 23.7% as calculated with the FFT system, and 43.0% ± 8.8% as calculated with the old system ($p < .05$, $N = 7$), the respective AUCs being 1,273.5 ± 461.1 percentage seconds and 616.5 ± 167.5 percentage seconds ($p < .05$). The tendency toward a stronger increase of mucociliary activity in the FFT analysis when the photoelectrical signal was considered poor, as compared to challenge during a better photoelectrical signal, was not significant according to the Mann-Whitney U-test for unpaired data.

Human Experiments. Recordings from the septal mucosa and those from aluminum foil on the nasal dorsum showed some similarities in the lower frequency spectrum (below 5 Hz). Typical FFTs of the signals are shown in Fig 5. The most likely explanation for the peak at approximately 1 Hz is the pulse beat, whereas peaks at 2 and 3 Hz may represent higher harmonics of the pulse. This was easily seen when using aluminum foil as a light-reflecting surface, each pulse beat causing vibrations in the foil. The major difference between the two recordings was seen between 7 and 10 Hz, where the septal recordings displayed distinct peaks less often noticed in the experiments with aluminum foil. In order to interpret the results, every FFT of raw data was analyzed stepwise and a histogram was calculated for each of the seven volunteers. The predominant peaks in the frequency intervals 0 to 5 Hz and 5 to 10 Hz (in one case, 5 to 20 Hz) were noted separately. Both frequency and the amplitude of the peak were calculated. It was found that the means were significantly higher for measurements in the human nasal mucosa: 7.8 ± 1.6 Hz (mucosa) versus 6.2 ± 0.4 Hz (aluminum) for frequencies above 5 Hz, and 2.9 ± 1.2 Hz (mucosa) versus 2.1 ± 1.0 Hz (aluminum) in the lower frequency interval. Raw data for this comparison are given in Table 2, and the corresponding histograms

TABLE 2. FREQUENCY MEANS OBTAINED BY ANALYSIS OF RECORDINGS OF MUCOCILIARY ACTIVITY IN HUMAN NASAL MUCOSA, COMPARED TO MEASUREMENTS FROM ALUMINUM FOIL PLACED ON NASAL DORSUM

	<5 Hz		*>5 Hz*	
Subject	*Mucosa*	*Aluminum*	*Mucosa*	*Aluminum*
1	4.06 Hz (30)	3.68 Hz (23)	11.25 Hz (31)	6.39 Hz (30)
2	3.96 Hz (31)	3.38 Hz (31)	6.82 Hz (31)	5.94 Hz (31)
3	2.10 Hz (30)	1.44 Hz (31)	6.64 Hz (31)	6.26 Hz (18)
4	1.07 Hz (30)	1.02 Hz (30)	8.46 Hz (30)	5.75 Hz (15)
5	3.74 Hz (31)	1.29 Hz (30)	7.00 Hz (28)	5.81 Hz (6)
6	3.46 Hz (31)	1.85 Hz (31)	7.07 Hz (31)	7.00 Hz (22)
7	1.89 Hz (30)	2.00 Hz (30)	7.18 Hz (29)	6.02 Hz (27)
Mean	2.90 Hz	2.09 Hz	7.77 Hz	6.17 Hz
SD	1.19 Hz	1.04 Hz	1.64 Hz	0.43 Hz

Frequency spectra below and above 5 Hz were analyzed separately. Intervals of 10 seconds during 5 minutes were analyzed in both types of experiments. Differences beween means were significant for peaks both above and below 5 Hz ($p < .05$, Wilcoxon's test for paired data). Figures in parentheses refer to number of distinct peaks seen.

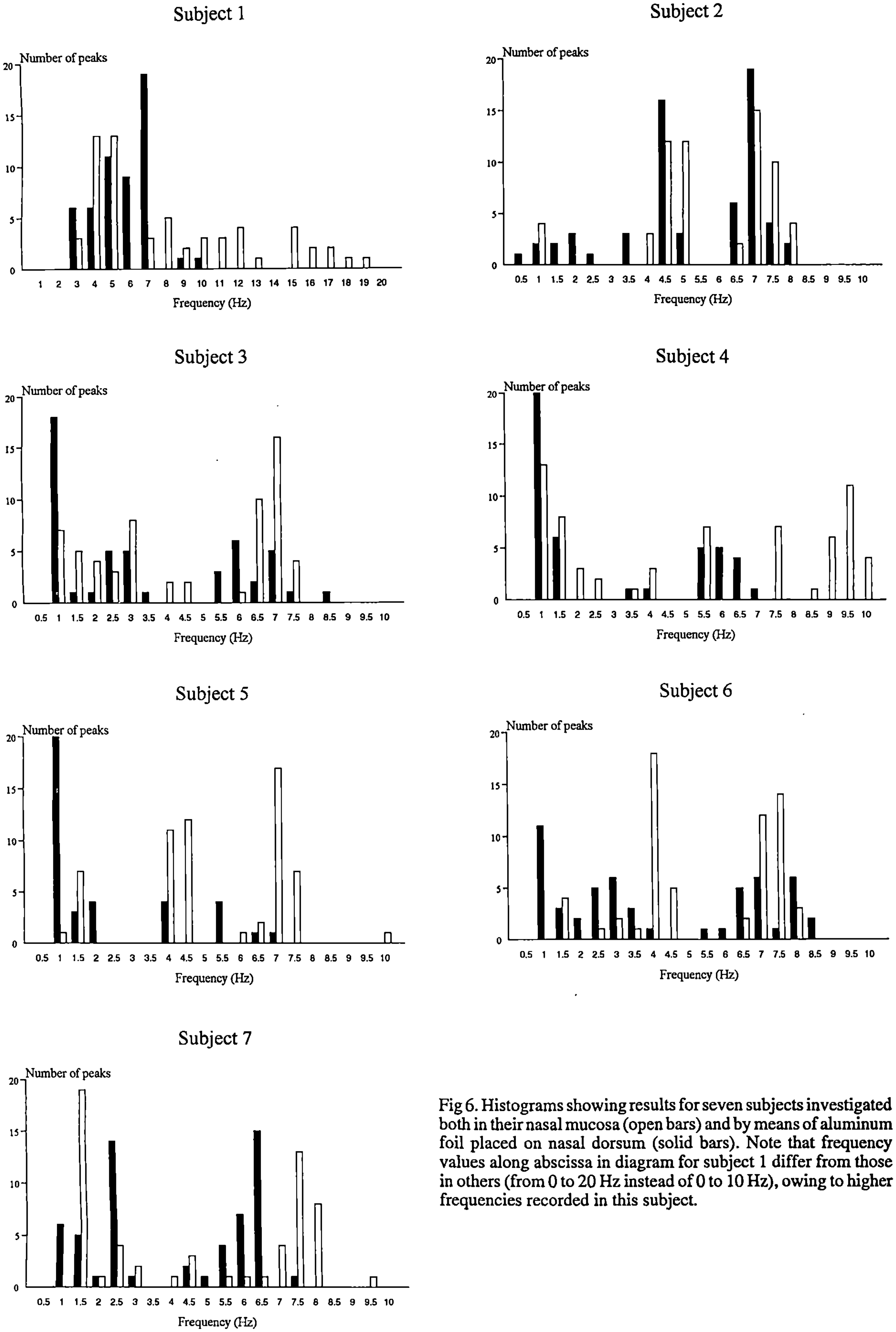

Fig 6. Histograms showing results for seven subjects investigated both in their nasal mucosa (open bars) and by means of aluminum foil placed on nasal dorsum (solid bars). Note that frequency values along abscissa in diagram for subject 1 differ from those in others (from 0 to 20 Hz instead of 0 to 10 Hz), owing to higher frequencies recorded in this subject.

for the number of peaks at each frequency in the subjects are shown in Fig 6.

Both similarities and dissimilarities were found between the two sources for the photoelectric signal. In one subject, No. 2, there was obvious overlapping between the aluminum and the intranasal recordings, whereas in subjects 1, 3, 4, 5, and 6 there was a clear-cut difference in the number of peaks in the region above 5 Hz. In subject 7, a number of peaks were found above 5 Hz in the recordings from the aluminum foil, approximately centered around 6.5 Hz. The number of peaks in the region above 5 Hz, in the intranasal measurements from the same subject, were centered around 7.5 Hz instead. In all subjects, intranasal peaks in the region above 5 Hz were found in almost all recordings during the 5-minute interval. This is in contrast to the recordings from the foil, which in subjects 3 to 6 showed distinct peaks above 5 Hz in a reduced number of measurements (range, 6 to 22 recordings; see Table 2).

There were no differences between the amplitudes of the FFT analysis, except for the aluminum experiments, in which the amplitude for peaks below 5 Hz was stronger than for peaks above 5 Hz: 2.3 ± 1.6 versus 0.6 ± 0.5 units ($p < .05$). In five of seven subjects, the ratio between the amplitudes above versus below 5 Hz was higher for the mucosal recordings than for the aluminum foil recordings, the respective mean ratios being 0.9 ± 0.8 and 0.7 ± 1.0 ($N = 7$; not significant).

DISCUSSION

Function Generator Experiments. The great difference in sensitivity between the FFT and old systems in these experiments is remarkable, but it should be borne in mind that the conditions during in vitro experiments, and in in vivo experiments on rabbits, admit relatively good signal-to-noise ratios, and the amplitude of the photoelectric signal can be kept well above the threshold for safe detection even with the old system. It is conceivable, however, that the limited sensitivity of the old system makes it less suitable for detecting mucociliary activity in human in vivo experiments, considering the presence of stronger artifacts and the inferior light situation that has to be dealt with. Therefore, the FFT system is believed to be better suited for experiments of this kind.

The reason for the poorer sensitivity of the old system is the peak-counting algorithm. When the signal is weak, the amplitude of the noise will be large relative to that of the LED signal. The computer program cannot distinguish between the LED signal and noise, but will count any peak that fulfils the criterion of the algorithm. In the algorithm, the difference in amplitude between two consecutive changes in signal direction is calculated and expressed as a percentage of the current envelope amplitude of the signal. When this ratio is sufficiently high, a peak will be counted. This means that noise will produce many peaks when the signal is weak. Accordingly, the output of the old system will be a frequency that is too high. Moreover, as the amplitude of the signal decreases, the dynamic range of the analog-to-digital conversion board will be less utilized. Only a limited number of the binary digits will be used, and the so-called quantization noise will increase. This will impair the behavior of both systems, but will be more conspicuous in the old system, because of the algorithm. As for the FFT system, the impaired signal-to-noise ratio affects the algorithm differently. If the noise can be described as random fluctuations in the signal, no individual frequency component of the noise will be particularly outstanding in the FFT, whereas the LED signal, which has a fixed frequency, will be easily distinguished. The difficulty for the old system in differentiating between noise and a weak signal was greater at frequencies below 15 Hz (Fig 2). Assuming the number of spurious peaks counted by the old signal processing system to be independent of the signal frequency, the contribution to the measured frequency by the spurious peaks will be relatively larger at lower frequencies.

Figure 3 shows that the FFT system easily detected frequencies of approximately 7, 10, and 15 Hz simulating beating cilia despite the contribution to the compound signal of stronger 1- and 4-Hz components representing artifacts, when the signals were presented simultaneously. The light intensity increased progressively as the current increased. Although, as expected, the plots resemble straight lines, the slopes were approximately 2.5, thus differing from the theoretic value of 2. This is probably to be explained by nonlinear characteristics of the LED. It was also found that the 1-Hz component was attenuated, as compared to the 4-Hz component, although both had the same intensity at the LED. The explanation of this is that the amplifier is AC-coupled in order to obstruct the DC component of the photoelectric signal, ie, the voltage that originates from the background light reflected from the mucosa. In fact, the AC coupling works as a high-pass filter. In our amplifier, as the cutoff frequency is approximately 3 Hz, the 1-Hz component of the compound signal is attenuated and thus less pronounced than the 4-Hz component in the FFT diagram. The amplitudes of all higher frequencies were approximately 10 times weaker than the amplitude of the 4-Hz component. This was to be expected, as the relationship between the LED current and FFT power is squared, and the voltage ratio is 1:3.

The FFT is a discrete Fourier transform that yields samples of the power spectrum at a limited number of frequencies. In the present FFT analysis of photoelectrical signals, there were 512 equally spaced frequencies ranging from 0 to 50 Hz. This entails a limitation in the FFT's ability to calculate the correct amplitudes of single-frequency signals, ie, sine waves. Only if the frequency of such a signal happens to be identical to one of the sample frequencies at which the FFT is computing will the amplitude be correctly calculated. At any other frequency, there will be leakage distributed among all other samples in the frequency domain, resulting in a loss of amplitude at the peak frequency. The maximum loss, which occurs when the frequency falls exactly in the middle between two frequency samples, is 3.9 dB, provided that a rectangular window function is used on the acquired data. When applying sinusoidal signals, it is therefore difficult to predict the exact amplitudes that will appear in the power spectrum, as they are strongly frequency-dependent. However, shifting the output frequencies from the function generator from 10 to 10.12 Hz, for example, affected the magnitude of the amplitudes, but not their dependency on the current delivered to the LED. As natural signals possess a wider frequency spectrum, the amplitude of a photoelectric signal containing real CBFs will not suffer from the above-mentioned limitations of the FFT.

Animal Experiments. The use of different algorithms in the two systems may explain why the respective frequency zero levels differ significantly from each other in some of the experiments. In the old system the mucociliary wave frequency is the number of peaks counted over a period of 10 seconds, then multiplied by six to obtain the number of waves per minute. As mentioned in Methods, the risk of counting peaks produced by noise is reduced by the computer program, but it is most probable that in spite of this, noise actually contributes to some extent to increase the value of mucociliary wave frequency, as the effect of noise cannot be completely eliminated by the old algorithm. Owing to the risk of counting noise peaks in the old system, the values may be smoothed; this may explain the lower coefficient of variation found when the old system was used for analysis of mucociliary wave frequency.

As, in the methacholine experiments, the old system yielded somewhat higher-frequency zero levels than did the FFT system, any percentage variation in CBF will be smaller with the old system than with the FFT system. This may account for some of the differences between the two plots in Fig 4, in which it can be seen that the increase according to the old system is smaller.

Correlation analysis showed the results obtained with the two systems to be strongly correlated. This indicates that changes in mucociliary wave frequency as calculated with the old system as percentages of the frequency zero levels correctly depict events in the mucociliary system in response to challenge, despite the old system's shortcomings. On the other hand, only the FFT system could detect any significant effect of a low dose of methacholine. This suggests that problems caused by noise, which are inevitable in all recordings of mucociliary activity, can be reduced by FFT processing of signals.

The results of experiments with morphometrically poor signals were noteworthy. The mean increase of the mucociliary wave frequency as a percentage of the frequency zero levels in these experiments did not differ from those in experiments with a signal considered to be optimal. This indicates that morphometric characteristics of the signal are of less importance than previously thought, and that weak, nonoptimal signals may contain information just as relevant as that obtainable from stronger light reflections considered optimal by the investigator.

Human Experiments. Assuming light reflections from aluminum foil placed on the nasal dorsum to display the same artifacts produced by pulse beat, breathing, and body tremor as do recordings from the nasal mucosa, the rationale for this experiment was that artifacts would be present in both measurements, but fluctuations of the light intensity due to ciliary beat would only be present in the recordings from the mucosa.

It was found in several of the subjects (Nos. 3 to 6) that there were fewer peaks in the FFT analysis in the interval above 5 Hz in signals from the aluminum foil than in signals from the nasal mucosa. In the FFT analysis, the mean amplitude was also lower in the interval above 5 Hz in the aluminum foil experiments; this is consistent with the finding that signals in the supposed ciliary region (between 5 and 20 Hz) were not only fewer but also weaker in these experiments.

In several of the subjects (1, 2, 5, and 6), a peak at 4 Hz appeared in recordings from the nasal mucosa. Whether some cilia actually beat that slowly in situ is not known. Using the laser light-scattering technique for recordings of the CBF in the trachea of dogs, Wong et al[5] found a frequency zero level of about 5 to 7 Hz in some dogs. On the other hand, in most studies of CBF in vitro, the frequency in human nasal cilia was found to be about 11 Hz by means of FFT.[2,3,11] Another possibility is that the 4-Hz peak actually represents the frequency of the metachronal wave, which may differ from the CBF. The frequency of the metachronal wave in humans is not known. In dogs and sheep, a metachronal wave period of 0.47 to 2.6 seconds, corresponding to a frequency of the metachronal wave of approximately 0.4 to 2 Hz, has been reported.[12] The observed 4-Hz peak is probably not to be explained by physiological tremor, as the frequency of this phenomenon is usually between 8 and 12 Hz, although in a recent study by others a slightly lower frequency of 6 to 9 Hz was found in healthy individuals.[13] However, in fatigue, another tremor may be seen in healthy individuals of about 4 to 6 Hz in addition to the physiological.[14] It is therefore possible that neck muscle tenseness may have produced the 4-Hz peak observed in these subjects, as sitting in a fixed position becomes awkward after a while for most volunteers.

When we used aluminum foil placed on the nasal dorsum as a light-reflecting surface, the photoelectric signal was affected by the pulse beat, each beat causing vibrations in the foil. These vibrations contain the fundamental frequency of the pulse, approximately 1 Hz, but also higher harmonics of which some, unfortunately, may appear in the frequency region in which mucociliary activity is expected to be manifested. In subjects 3 to 6, the spectra in the aluminum foil experiments seemed to reflect the pulse beat, with a predominant peak close to 1 Hz, whereas in subjects 1 and 2 the spectra did not manifest this expected pattern. A possible explanation is that the mechanical effect of the pulse beat varies from one subject to another because of individual variations in the distance between the pulsating blood vessel and the mucosa. In those subjects in whom the pulse beat obviously strongly affected the signal, the higher harmonics of the pulse beat might have been falsely "interpreted" by the FFT system as frequencies produced by cilia. In interpreting the frequency histograms shown in Fig 6, it should be borne in mind that frequencies below 3 Hz are attenuated by filters, which means that the fundamental amplitudes of the pulse beat are actually stronger than they appear in the histograms.

Previous experiments in vitro on explants from the human nasal mucosa have shown the mean mucociliary frequency before challenge to be 10 to 11 Hz.[2,3,11] It is noteworthy that the in vivo experiments yielded a lower frequency, with a mean of 7.8 Hz. At present there is no unequivocal explanation of this discrepancy. It seems reasonable, however, that the mucus, which is absent in the in vitro experiments, to some extent impedes the movement of the cilia — hence the higher frequency in in vitro experiments. It is also possible that other factors, such as release of inflammatory mediators by the biopsy procedure, accelerate mucociliary activity in the in vitro specimens. Indeed, such important inflammatory mediators as prostaglandins are potent ciliary stimulators,[15] and trauma has been shown to profoundly increase the mucociliary wave frequency in the canine trachea.[7]

The results of the human in vivo experiments suggest that the quality of the photoelectrical signal can be impaired by artifacts caused by pulse beat, breathing, and body tremor. In the worst scenario, the predominant frequency of the power spectrum may derive solely from such artifacts, thus causing an erroneous reading of the mucociliary wave frequency. The present findings show that the sources of the artifacts have to be identified. One prerequisite is to analyze the photoelectric signal in the frequency domain. The FFT, which has been employed in this study, is a tool that can be used also in the process of identifying the origins of the artifacts. Moreover, the function generator experiments showed the FFT system to be able to correctly calculate a simulated mucociliary wave frequency at much lower signal-to-noise ratios than the old system. We expect to benefit from these features of the FFT method, particularly in future human in vivo experiments, in which the signal-to-noise ratios are lower and artifacts more frequent than in other types of experiments.

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REFERENCES

1. Jafek BW. Ultrastructure of human nasal mucosa. Laryngoscope 1983;93:1576-99.

2. Phillips PP, McCaffrey TV, Kern EB. Measurement of human nasal ciliary motility using computerized microphotometry. Otolaryngol Head Neck Surg 1990;103:420-6.

3. Ingels KJAO, Meeuwsen F, van Strien HLCJ, Graamans K, Huizing EH. Ciliary beat frequency and the nasal cycle. Eur Arch Otorhinolaryngol 1990;248:123-6.

4. Hybbinette JC, Mercke U. A method for evaluating the effect of pharmacological substances on mucociliary activity in vivo. Acta Otolaryngol (Stockh) 1982;93:151-9.

5. Wong LB, Miller IF, Yeates DB. Stimulation of ciliary beat frequency by autonomic agonists: in vivo. J Appl Physiol 1988;65:971-81.

6. Lindberg S, Dolata J, Mercke U. Nasal exposure to airway irritants triggers a mucociliary defence reflex in the rabbit maxillary sinus. Acta Otolaryngol (Stockh) 1987;104:552-60.

7. Wong LB, Yeates DB. Stimulation of tracheal ciliary beat

frequency by localized tissue incision. J Appl Physiol 1990;68: 411-6.

8. Lindberg S, Runer T. Method for in vivo measurement of mucociliary activity in the human nose. Ann Otol Rhinol Laryngol 1994;103:558-66.

9. Toremalm NG, Håkansson CH, Mercke U, Dahlerus B. Mucociliary wave pattern. An analysis of surface light reflections. Acta Otolaryngol (Stockh) 1974;78:247-52.

10. Hybbinette JC, Mercke U. Effects of the parasympathomimetic drug methacholine and its antagonist atropine on mucociliary activity. Acta Otolaryngol (Stockh) 1982;93:465-73.

11. Roth Y, Aharonson EF, Teichtahl H, Baum GL, Priel Z, Modan M. Human in vitro nasal and tracheal ciliary beat frequencies: comparison of sampling sites, combined effects of medication, and demographic relationships. Ann Otol Rhinol Laryngol 1991;100:378-84.

12. Wong LB, Miller IF, Yeates DB. Nature of the mammalian ciliary metachronal wave. J Appl Physiol 1993;75:458-67.

13. Comby B, Chevalier G, Bouchoucha M. A new method for measurement of tremor at rest. Arch Int Physiol Biochim Biophys 1992;100:73-8.

14. Lippold O. The tremor in fatigue. Ciba Found Symp 1981;82:234-48.

15. Dolata J, Lindberg S, Mercke U. The effects of prostaglandins E1, E2 and F2 alpha on mucociliary activity in the rabbit maxillary sinus. Acta Otolaryngol (Stockh) 1989;108:290-7.

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EUROPEAN WORKING GROUP ON GENETICS OF HEARING IMPAIRMENT

The Second HEAR Project Workshop will be held in Milan, Italy, October 11-12, 1996. For further information and submission of abstracts, please contact: Alessandro Martini, MD, Professor of Audiology, Servizio di Audiologia, Clinica ORL, Università degli Studi, Corso Giovecca 203, I-44100 Ferrara, Italy; telephone: +39 532 295429 or 295341; fax: +39 532 295887; E-mail: mma@ ifeuniv.unife.it

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HISTORICAL VIGNETTE:SIR WILLIAM HINGSTON, NASAL FIBROMAS, AND THE UPSIDE-DOWN OPERATION

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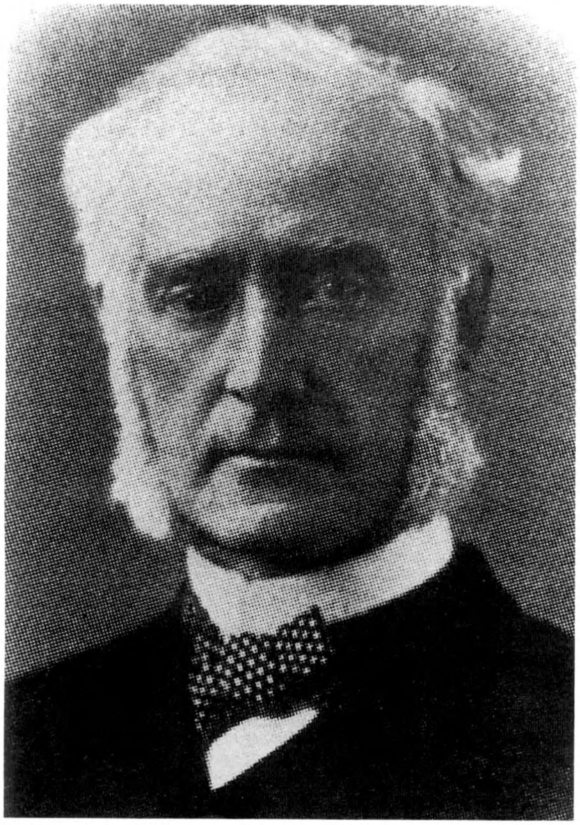
Dr William Hingston was one of Canada's most illustrious surgeons in the second half of the 19th century. Some interesting accounts are presented of his experience with "fibrous nasopharyngeal tumors" and the ingenuity he used to resect these difficult tumors.

KEY WORDS — nasal fibroma, William Hingston.

Dr William Hingston (see Figure) was one of Canada's most illustrious surgeons in the second half of the 19th century. He was born in Lower Canada in 1829 and received his medical degrees at McGill University and Edinburgh. This was followed by a year visiting the clinics of several European cities. Upon his return to Canada in 1854, he was accepted on staff at the Hôtel-Dieu Hospital in Montreal, where he continued an active surgical practice until the early years of the 20th century. He wrote many medical articles and was the author of a book on the climate of Canada.[1] Dr Hingston found the time to be a member of many scientific and other organizations, was president of a bank and director of several companies, and in 1875-1876 even served a term as mayor of Montreal. In recognition of his many talents, he was knighted by Queen Victoria in 1895 and appointed to the Canadian Senate in 1896.[1]



Dr William Hingston. (Reprinted with permission from reference 1, page 69, from the Collection des Religieuses Hospitalières de Saint-Joseph de Montréal.)

Dr Hingston performed much surgery of the head and neck. He was adept at maxillectomies and mandibulectomies, and was the first surgeon to resect both the mandible and tongue for carcinoma. Even though he was the earliest to publish such a case, he never received the credit for it, probably because it was published in a Canadian journal not read in other countries.[1]

One day in 1889, a patient with an interesting problem came to see him.[2] A young man, otherwise in good health, had a large tumor of the nasal airway. Anteriorly, it protruded through the nostrils, dilated the alae, and distended the nasal bones, giving the patient a froglike appearance. Posteriorly, it had deformed the hard palate and stretched the soft palate, and was protruding one half inch below it. The patient could not breathe at all through his nose, and had developed considerable dysphagia. Digital examination revealed that the tumor was not a nasal polyp or polyps on a narrow stalk, but was probably a tumor adherent to the periosteum of the base of the occiput, the atlas, and the sphenoid. Dr Hingston felt that it represented a nasopharyngeal polyp of the fibromatous type (probably a nasopharyngeal fibroma in modern terminology).

Dr Hingston began to think about the best way to remove this mass. From his experience with these tumors, he knew that the bleeding would be considerable. He recalled one case that he started using chloroform anesthesia. The approach was an incision through the middle of the upper lip, along one alar base and up the side of the nose, then cutting bone

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deeper in order to retract the nose onto the opposite cheek. As he proceeded to resect the tumor, the bleeding was alarming. There was so much hemorrhage that Dr Hingston felt the patient would drown in his own blood. He called for four volunteers. (As he was an interesting teacher as well as an excellent surgeon, the amphitheater of his operating room was always full of spectators.) These came down. Two of them grabbed the legs, and the other two the trunk and upper extremities, holding the patient upside down, with the head gently touching the floor. Dr Hingston then completed the tumor resection, controlled the bleeding, and closed the incisions while kneeling.

That must have been a sight to behold! Although he did not mention it, the anesthetist probably had to give additional anesthetic from time to time, and an assistant may have been present to hold the nose out of the way. The two or three of them performed their duties on their knees in an ever-enlarging pool of blood, with four men straining to hold the patient steady in the upside-down position. Not only was the recovery uneventful, but the patient ended up with a barely visible scar.[2]

Dr Hingston further thought about ways to reduce the bleeding. He recalled another patient he had operated on several years before. A visiting American surgeon, after examining the patient and seeing Dr Hingston's long hands, remarked that with his strong hands and long fingers, Dr Hingston should use a method utilized by Dr Gross of Philadelphia — blunt digital dissection. (Dr Samuel Gross [1805-1884] was perhaps the greatest American surgeon of his time. He was a professor of surgery in Louisville, Ky, from 1840 to 1856, and at the Jefferson Medical College, Philadelphia, Pa, from 1856 to 1882.) Dr Hingston tried this and found that it worked well, but that his fingers were sore after the surgery. On another occasion, his fingers were so painful after the operation that he could not use his hands properly for 2 weeks.

After all his deliberations, Dr Hingston decided that the safest way to remove the tumor of his patient would be to have him in the sitting position in a chair, and to perform the procedure without anesthesia. (Local anesthesia was still experimental at this time.) This would allow the patient to continually cough up the blood. Blunt digital dissection would be used. Several stalwart assistants held the patient in the chair. A thick suture was placed through the tumor in the pharynx and given to an assistant to apply mild traction.

During the initial stages of surgery, the patient sat quietly. As Dr Hingston began to separate the tumor using his fingers, the patient endured the pain at first but soon began to struggle violently, requiring all the strength of the assistants to hold him in the chair. Dr Hingston calmed him with some encouraging words, introduced a mouth gag, and continued the operation. The patient bore the rest of the operation stoically without moving. The bleeding was massive. The patient lost consciousness *"plus d'une fois"* (more than once), but revived each time to cough up the blood.

At one stage Dr Hingston thought the patient would not survive the procedure because of the severe hemorrhage. However, he did survive, and felt so much better the day after the surgery that he refused to stay longer in the hospital and was allowed to leave.

REFERENCES

1. Meunier P. La chirurgie à l'Hôtel-Dieu de Montréal aux XIXe siecle. Montreal, Canada: Les Presses de l'Université de Montréal, 1989.

2. Clinique Chirurgical Hôtel-Dieu de Montréal — par M. le Dr. Hingston. Tumeurs fibreuses naso-pharyngiennes. Notes recueilles par J.L.G. Gaz Med Montreal 1889;3:249-52.

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IMAGING CASE STUDY OF THE MONTH

SCHWANNOMA OF THE BRACHIAL PLEXUS

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CASE REPORT

A 41-year-old woman was referred to the Department of Otorhinolaryngology–Head and Neck Surgery, Inselspital Bern, for evaluation and treatment of a neck mass on the left side. In 1969, her left cervical and mediastinal lymph nodes were irradiated for Hodgkin's lymphoma, which recurred in 1972 in the retroperitoneal lymph nodes. Treatment by splenectomy, irradiation, and chemotherapy followed. In 1982 a right thyroidectomy, a left partial thyroidectomy, and radioiodine treatment were performed because of papillary thyroid carcinoma.

During the subsequent 12 years, the patient was well and without any sign of recurrence until November 1994, when a left neck mass was observed. The results of the head and neck examination showed a 2 × 3-cm firm, immobile left posterior cervical mass. Palpation produced a tingling sensation over the distribution of the radial nerve. The results of the remainder of the physical examination, especially the neurologic examination, were within normal limits.

Magnetic resonance imaging (MRI) demonstrated a tumor of the C-6 root of the brachial plexus (see Figure). The axial, nonenhanced T1-weighted spin echo image (T1SE) demonstrated a well-delineated mass with smooth margins situated posterior to the left carotid artery and lateral to the left intervertebral foramen C5-6 (see Figure, A). On the corresponding T2-weighted spin echo image (T2SE), the tumor showed a very high signal intensity (see Figure, B); after intravenous administration of contrast material, a strong peripheral enhancement was observed (see Figure, C). The central part of the fusiform-shaped tumor showed areas of cystic degeneration. On the basis of these findings the diagnosis of a neurogenic tumor of the C-6 left nerve root was made. Fine needle aspiration cytology was suggestive of schwannoma.

Neck exploration was undertaken by the head and neck surgeon with the neurosurgeon in attendance. A fusiform tumor was found involving the C-6 nerve root, extending to the outer lateral border of the foramen. The tumor was resected in toto with preservation of the nerve root by means of microsurgical techniques. Histology of the surgical specimen confirmed the diagnosis of schwannoma. Postoperatively, the patient had decreased strength of the wrist extensors and elbow flexors. At 12 months, these symptoms had nearly disappeared, and total return of function was attained.

DISCUSSION

Primary tumors of the brachial plexus are unusual. In 1987 Lusk et al[1] summarized 147 cases published since 1886. During the 20-year period of 1948 through 1967, 23 cases of brachial plexus tumors were observed at the Mayo Clinic.[2] Verocay[3] is credited with the first description of a nerve tumor as a special entity in 1910. Two types of peripheral nerve tumors must be distinguished: schwannoma, which grows extrinsic to its parent fascicles, and neurofibroma, not encapsulated, intertwining itself within its fascicles of origin. Lusk et al[1] stated that the perineural fibrocytes give rise to the more invasive neurofibromas, and Schwann cells, which seem to be highly differentiated perineural cells, give rise to schwannomas. Microscopically, schwannomas are composed of densely packed spindle cells (the so-called Antoni type A) or more loosely textured myxoid stroma (Antoni type B).[4] Neurofibromas are often associated with von Recklinghausen's disease. Several cases of schwannomas associated with thyroid tumors, as in our case, are reported in the literature.[5,6] Symptoms of numbness, paresthesia, and shooting pain on palpation associated with upper extremity weakness are characteristic of these lesions. However, half of the patients have no symptoms of neural impairment.[7] The differential diagnosis must include lymphadenopathy, lipomas, angiomas, and uncommon tumors of this area, such as brachial cleft cysts and vascular anomalies.[8] The pathologic diagnosis can be made by needle aspiration cytology or histology prior to sur-

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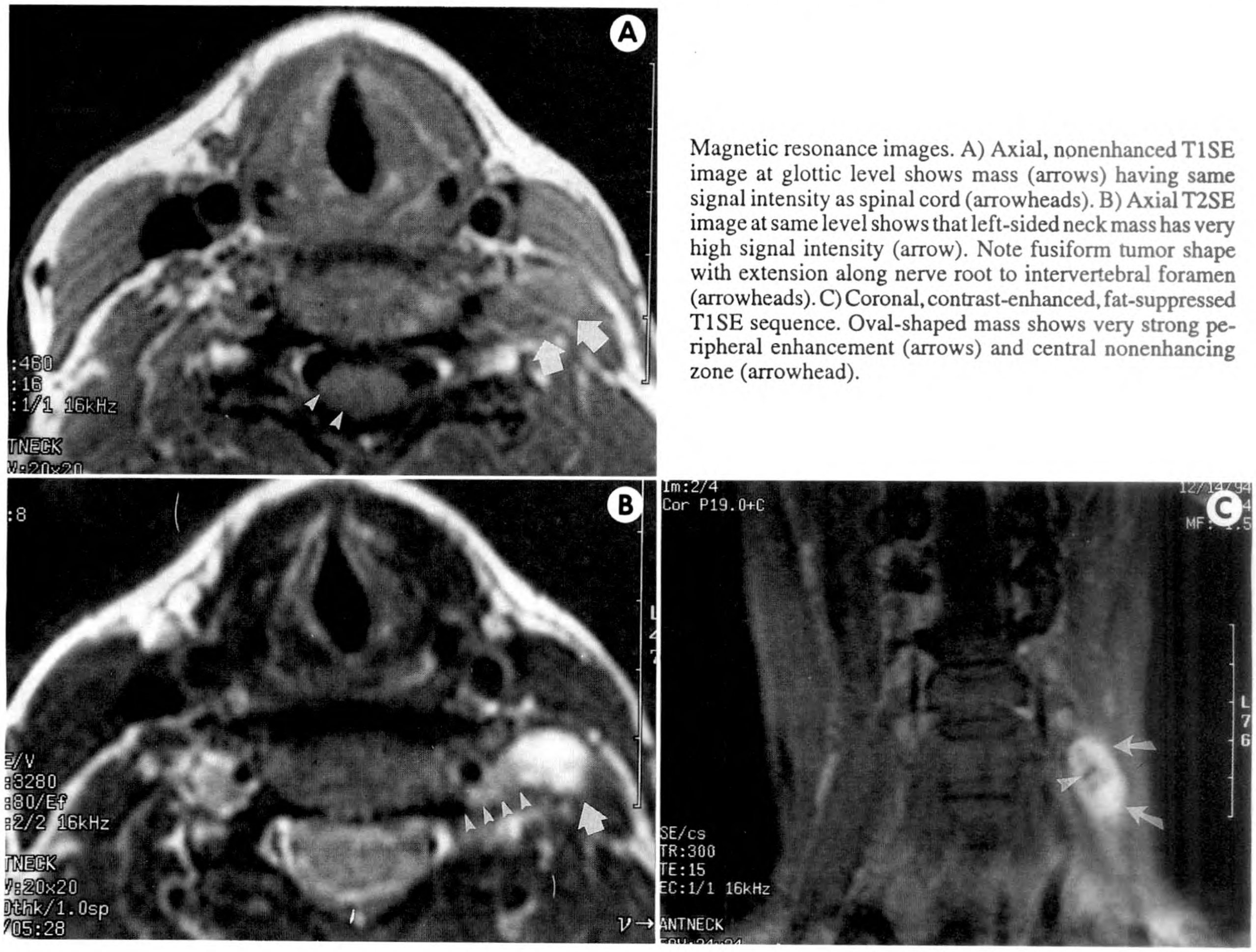

Magnetic resonance images. A) Axial, nonenhanced T1SE image at glottic level shows mass (arrows) having same signal intensity as spinal cord (arrowheads). B) Axial T2SE image at same level shows that left-sided neck mass has very high signal intensity (arrow). Note fusiform tumor shape with extension along nerve root to intervertebral foramen (arrowheads). C) Coronal, contrast-enhanced, fat-suppressed T1SE sequence. Oval-shaped mass shows very strong peripheral enhancement (arrows) and central nonenhancing zone (arrowhead).

gical treatment, as was done in our case. One must be aware that the aspiration biopsy technique has inadequacies in dealing with these pleomorphic tumors.[7] A false malignant diagnosis can lead to large, unnecessary en bloc resections of benign lesions, with severe postoperative neural deficits.[5] Treatment of these tumors is by surgical excision with microsurgical techniques. The schwannomas can usually be removed without or with only temporary postoperative weakness of several parts of the arm, as in our case. Excision of neurofibroma, occurring within the fascicles themselves, frequently leads to an inevitable neurologic deficit.

Schwannomas and neurofibromas display typical findings on MRI. Nearly 75% of nerve root tumors have the same signal intensity as the spinal cord on T1SE images.[9] More than 95% have a very high signal intensity on T2SE sequences, and nearly all nerve sheath tumors enhance following contrast administration.[9,10] In schwannoma, there is no direct correlation between Antoni A and Antoni B tissue types and features recognized on MRI scans.[11] On histology, approximately 40% of spinal schwannomas have a cystic component.[10] A "target" appearance (hyperintense rim and hypointense center) is often seen on contrast-enhanced T1SE images in both schwannomas and neurofibromas. Cystic or necrotic degeneration is responsible for the "target" appearance on MRI. In our case, all imaging findings (signal intensities on T1SE and T2SE sequences, as well as cystic degeneration) were typical for a neurogenic tumor.

In addition, on the axial images, the typical fusiform tumor shape, as well as extension up to the lateral border of the intervertebral foramen, very strongly suggested a tumor of the C-6 nerve root.

REFERENCES

1. Lusk MD, Kline DG, Garcia CA. Tumors of the brachial plexus. Neurosurgery 1987;21:439-53.
2. Dart LH, MacCarty CS, Love JG, Dockerty MB. Neoplasms of the brachial plexus. Minn Med 1970;53:959-64.
3. Verocay J. Zur Kenntnis der Neurofibrome. Beitr Pathol Anat Allg Pathol 1910;48:1-68.
4. Varma DGK, Moulopoulos A, Sara AS, et al. MR imaging of extracranial nerve sheath tumors. J Comput Assist Tomogr 1992;16:448-53.
5. Noterman J, D'Haens J, Nubourgh Y, Colle H. Les tremeurs

des plexus brachial. Neurochirurgie 1982;28:139-41.

6. Goodwin JT. Encapsulated neurilemoma of the brachial plexus. Cancer 1952;5:708-20.

7. Fisher RG, Tate HB. Isolated neurilemomas of the brachial plexus. J Neurosurg 1970;32:463-7.

8. Handler SD, Canalis RF, Jenkins HA, Weiss AJ. Management of brachial plexus tumors. Arch Otolaryngol 1977;103:653-7.

9. Hu HP, Huang QL. Signal intensity correlation of MRI with pathological findings in spinal neurinomas. J Comput Assist Tomogr 1992;34:98-102.

10. Ischii N, Matsuzava H, Houkin K, et al. An evaluation of 70 spinal schwannomas using conventional computed tomography and magnetic resonance imaging. Neuroradiology 1991;33:542-5.

11. Demachi H, Takashima T, Kadoya M, et al. MR imaging of spinal neurinomas with pathological correlation. J Comput Assist Tomogr 1990;14:250-4.

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OTOLARYNGOLOGY UPDATE

The University of California Otolaryngology Update 1996 will be held in San Francisco, California, November 7-10, 1996. For more information, please contact University of California, Office of Continuing Medical Education, 1855 Folsom St, MCB-630, Box 0742, San Francisco, CA 94143-0742; telephone (415) 476-4251; fax (415) 476-0318.

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CLINICOPATHOLOGICAL CONSULTATION

HODGKIN'S DISEASE: OLD AND RECENT CLINICAL CONCEPTS

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The diagnosis of Hodgkin's disease (HD) is still primarily based on the histopathologic criteria presented by Lukes et al in 1965. Over the past three decades, however, advances in immunohistochemistry, molecular studies, and virologic findings have provided evidence that HD exhibits significant heterogeneity. Whereas the nodular sclerosis subtype is the major subset of the "classic" disease, it has become evident that the nodular, lymphocytic predominance subtype may represent a minor distinct subset, and possibly a distinct disease entity. Furthermore, there is strong evidence that the mixed cellularity subtype may be an Epstein-Barr virus–related disorder. Finally, based on phenotypic and genotypic features, the existence of a "continuum" between a fraction of HD cases, including the lymphocytic depletion subtype, and CD30-positive anaplastic large cell lymphomas has been suggested.

INTRODUCTION

In recent years, the concept that Hodgkin's disease (HD) is a malignant disorder has been widely accepted among pathologists, clinicians, and researchers working on this disease. However, the nature and origin of the Reed-Sternberg (RS) cell, the presence of which is essential to the diagnosis of HD, remains a matter of controversy, in spite of the most recent and sophisticated investigations.

After the redefinition of the pathologic picture of HD in the 1960s, most investigators focused their attention on non-Hodgkin's lymphomas. In the last decade, however, immunohistochemical and molecular findings have revived interest in HD. This review will examine recent concepts and diagnostic features of the disease.

PATHOLOGIC FEATURES

The classification of HD widely adopted among pathologists and clinicians is the one presented at the meeting on HD in Rye, NY, in 1965.[1] The Rye classification combined the six groups of the Lukes et al[2] classification to produce four main histologic subtypes with lymphocytic predominance (LP), nodular sclerosis (NS), mixed cellularity (MC), and lymphocytic depletion (LD).

The Lukes et al[2] classification was based on the quantitative relationship between lymphocytes and diagnostic RS cells (LP versus LD), and the different types of connective tissue proliferation (NS versus LD; diffuse fibrosis), with the MC subtype representing those cases that failed to fit into the other subtypes. This classification further included the definition of three (nondiagnostic) RS cell variants associated with specific histologic subtypes of the disease: L & H (lymphocytic and histiocytic) cells, associated with LP; lacunar cells, detectable in NS; and the pleomorphic type, seen in LD. The mononuclear (Hodgkin's cell) variant in an appropriate cellular background was considered as merely suggestive, but not diagnostic, of HD.

The pathologic criteria identifying the histologic subtypes of HD have not changed since they were first described.[1,2] It has been stressed,[3] however, that familiarity with the terminology of the original Lukes et al[2] classification facilitates recognition of the different variants, so the latter terms are useful for pathologists who can translate them into those of the Rye classification.[1]

Clinical and pathologic experience over the past two decades, and recent advances in immunohistochemistry and molecular studies, have indicated that HD exhibits significant heterogeneity.[4-10] Different HD subsets have been recognized that may reflect evolutional forms of a single disease or separate, although possibly closely related, diseases.[11] A major subset includes the NS subtype; the second subset encompasses almost the entire spectrum of the non-NS subtypes. It mainly includes the MC subtype, along with a smaller group of related cases with features of the LP-diffuse and LD subtypes. The cellular-phase NS variant, showing lacunar RS cells

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MORPHOLOGIC, IMMUNOPHENOTYPIC, AND VIROLOGIC FEATURES OF LP HD VERSUS CLASSIC HD

	LP HD	*Classic HD*
Atypical cells	LP variants (L & H)	Diagnostic RS cells
Diagnostic or lacunar RS cells	Rare to absent	Present
CD15	Negative	Positive
CD30	Usually negative	Positive
CD20	Usually positive	Usually negative
CD45	Positive	Usually negative
EMA	Positive	Negative
EBV (in large cells)	Usually negative	Often positive (20% to 70%)

LP HD — lymphocyte predominance Hodgkin's disease, classic HD—Hodgkin's disease with nodular sclerosis and mixed cellularity, RS—Reed-Sternberg, EMA—epithelial membrane antigen, EBV—Epstein-Barr virus.

with no evidence of sclerosis,[12,13] may belong to this subset. The third, minor subset includes the nodular LP subtype. Sufficient evidence has emerged to warrant recognition of this subtype of HD as a distinct entity. It differs morphologically, immunophenotypically, and clinically (see below) from classic HD.[7,14,15] Typically, in the nodular LP subtype, progressively transformed germinal centers are seen in the same or other nodes, while diagnostic RS cells are rare or absent (see Table).

ANATOMIC SITES OF INVOLVEMENT AND CLINICAL FEATURES

There are differences between HD and the non-Hodgkin's lymphomas regarding the anatomic sites of involvement at presentation. In HD the rarity of any initial involvement of Waldeyer's ring, skin, gastrointestinal tract, or mesenteric lymph nodes is well known,[16] while these sites are often involved by non-Hodgkin's lymphomas.[17]

Among the HD subtypes, LP usually presents as an enlarged cervical lymph node,[18] whereas the NS subtype more commonly involves the mediastinum than all the other subtypes combined.[19] The LP subtype of HD occurs at all ages and it usually involves peripheral lymph nodes, sparing the mediastinum. Survival is long, with or without treatment, for localized cases. The disease may progress to large B-cell lymphoma. The NS variant is most common in young adults; the mediastinum is commonly involved; this variant is often curable. Patients with the MC subtype are usually adults and the stage may be more advanced than in the NS or LP types, involving the lymph nodes, spleen, liver, or marrow. The course is moderately aggressive. The LD subtype is the least common variant of HD and usually occurs in older people, in human immunodeficiency virus (HIV)–positive individuals, and in nonindustrialized countries. It frequently presents with abdominal lymphadenopathy and spleen, liver, and bone marrow involvement, without peripheral adenopathy. The stage is usually advanced at diagnosis; response to treatment is reportedly no different from that of the other subtypes, however.[20]

HODGKIN'S DISEASE AND HIV INFECTION

Although several reports have suggested an association of HD with the acquired immunodeficiency syndrome (AIDS),[21,22] HD occurring in patients with HIV infection is not yet considered an AIDS-defining condition.

In the largest registry linkage study,[23] solid tumors as a whole did not occur more often than expected among persons with AIDS, but two neoplastic forms were found to be significantly increased: HD and carcinomas of the anorectum. The high rate of HD in this population-based cohort of patients with AIDS strongly supports the previous reports[21,22,24] that suggested that at least a subset of HD may be part of the AIDS presentation. In fact, the findings of recent studies have documented a significant difference in the distribution of MC and LD subtypes of HD in HIV-infected patients as compared with HD in the general population.[24-27] Accordingly, HD has been described in patients with HIV infection in association with unfavorable prognostic factors, such as advanced stage at presentation, unfavorable histologic subtypes, and an aggressive clinical course.[28-30] Furthermore, a pathogenic role of Epstein-Barr virus (EBV) in a large proportion of HD occurring in the HIV setting has been assumed on the basis of the higher frequency of EBV genome demonstration.[31-33]

Most of these clinicopathologic and virologic characteristics have recently been studied in a large group of patients with HIV infection collected within the framework of the GICAT (Gruppo Italiano Cooperativo AIDS e Tumori).[34] In 114 patients with HD and HIV infection compared with a single-institution series of 104 HIV-negative patients with HD, this study revealed a younger median age (29 versus 38 years), a male prevalence (90% versus 56%), and a higher percentage of stage IV disease (52% versus 15%), B (clinical) symptoms (77% versus 35%), and extranodal disease (63% versus 29%) at presentation. Seventy-nine percent of the 114 patients were intravenous drug users, and 17% had AIDS diagnosed prior to HD onset; the median CD4+ cell count at diagnosis of HD was 275/mm^3. In the same study the relationship between EBV and HD in the HIV setting

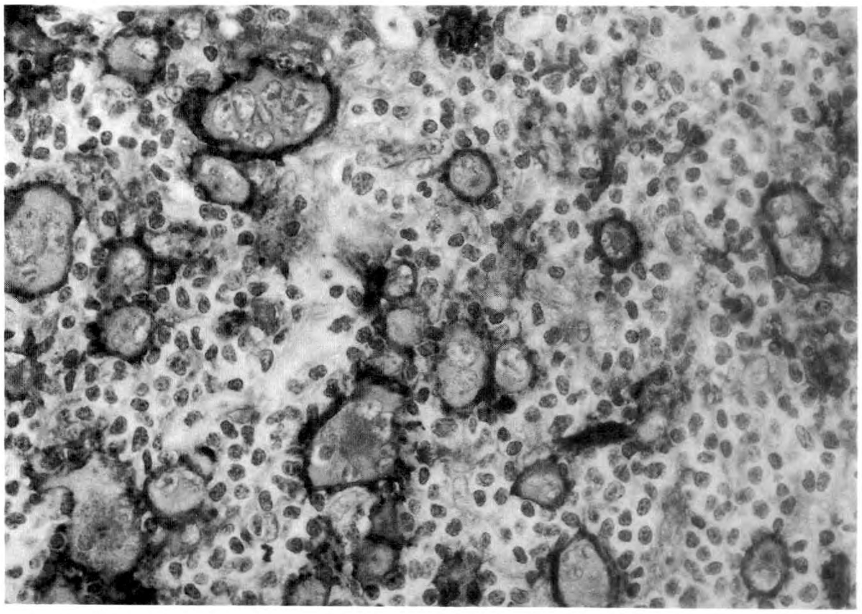

Fig 1. Hodgkin's disease, nodular sclerosis subtype (Bouin-fixed, paraffin-embedded tissue section; alkaline phosphatase–anti–alkaline phosphatase [APAAP] method; hematoxylin counterstain; original ×400). Immunostaining with anti-CD40 monoclonal antibody 89 discloses presence of population of large Reed-Sternberg cells that are characterized by strong membrane labeling.

was determined in 18 cases by an in situ hybridization technique, immunostaining for EBV-encoded latent membrane protein–1 (LMP-1) expression, and the Southern blot technique. Such a combined approach revealed that 14 (78%) of the 18 HD patients with HIV infection had associated EBV — a frequency significantly higher than is observed in HD cases without HIV in a single-institution series (27/104 or 26% by immunohistochemical analysis for LMP-1, and 12/44 or 27% by Southern blot analysis). The finding that monoclonal EBV genomes were detected in all EBV-positive cases and that the same EBV-infected clone was present in multiple metachronous locations of the disease provided further evidence to support the causal involvement of EBV in HIV-related HD.

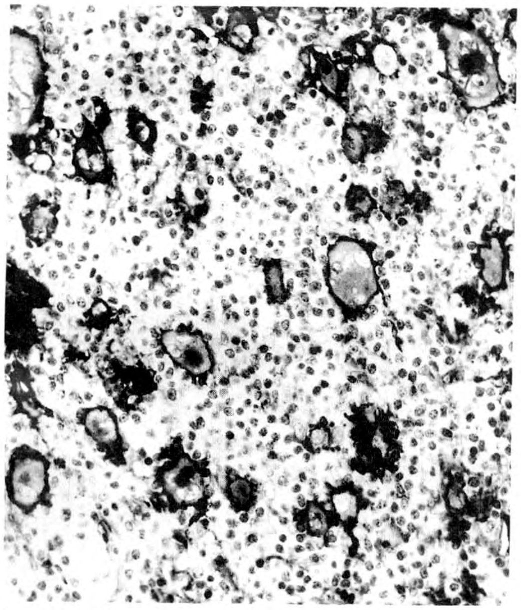

Fig 2. Hodgkin's disease, nodular sclerosis subtype (Bouin-fixed, paraffin-embedded tissue section; APAAP method; hematoxylin counterstain; original ×320). Many Reed-Sternberg cell variants (lacunar cells) are seen in this field; most cells show distinct pattern of staining for anti-CD40 antibody 89; strong membrane staining is associated with dotlike cytoplasmic positivity.

IMMUNOPHENOTYPING

Extensive immunophenotypic analysis has shown heterogeneous antigenic profiles on RS cells,[35] and there have been no convincing indications of RS cell–specific antigens. On the basis of such evidence, a combination of several markers, including activation antigens (CD30, CD15, CD25, CD71), HLA-related molecules (CD74, HLA-DR), T and B cell–associated antigens, and leukocyte common antigen, is currently employed for the immunodiagnosis of HD in routine pathology practice.[7,36-39] The nodular variant of the LP subtype of HD poses special diagnostic problems, since L & H cells usually express several B-cell markers,[7,14,38,40] but lack consistent staining with anti-CD15[40-42] and anti-CD30[7] monoclonal antibodies (MoAbs; see Table).

It has recently been reported[43] that RS cells and their morphologic variants expressed CD40 in 139 of 139 HD cases, irrespective of the histologic subtype or their antigenic phenotype (B, T, non T–non B). The staining of RS cells with anti-CD40 MoAb 89 was very intense on the cell membranes (Fig 1), being associated with cytoplasmic reactivity and a strong dotlike pattern in the paranuclear area (Fig 2). Immunodetection of CD40 on Bouin or formalin-fixed paraffin-embedded material therefore appeared of considerable value in identifying RS cells, including those of the nodular variant of the LP subtype.

Expression of CD30 is not restricted to the RS cells

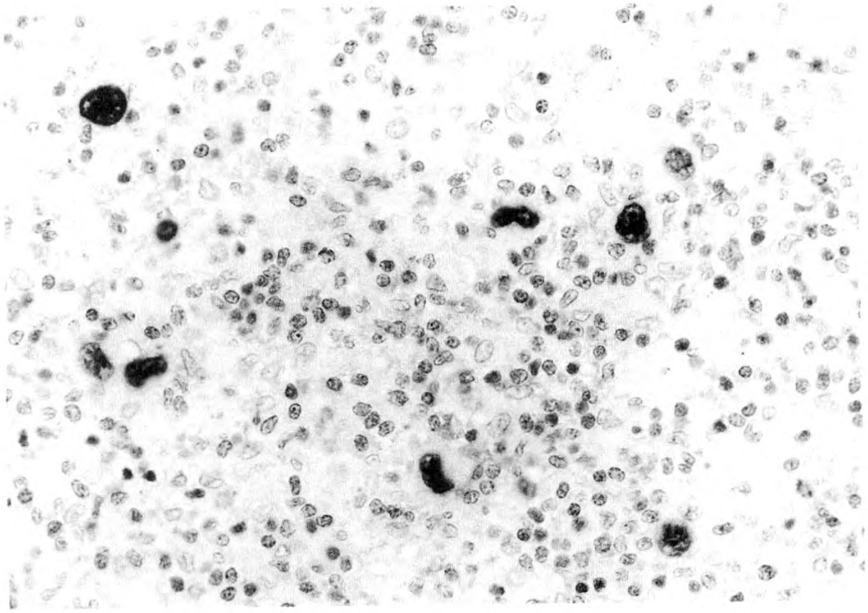

Fig 3. EBER in situ hybridization (ISH) performed in Epstein-Barr virus (EBV)–positive Hodgkin's disease of mixed cellularity subtype (Bouin-fixed, paraffin-embedded tissue section; ISH; nuclear fast red counterstain; original ×400). Signal is present as dense grains over nuclei of Reed-Sternberg cells.

of HD, as CD30 MoAbs also identify a group of non-Hodgkin's lymphomas designated anaplastic large cell (ALC) lymphomas.[36] In most cases, CD30-positive ALC lymphomas (first described as a distinct pathologic entity in 1985[44]) exhibit a considerable morphologic resemblance to HD.[45-47] Both conditions share several phenotypic and genotypic features, and the existence of a *continuum* between these two neoplastic lesions has been suggested.[48] The overlapping phenotypic features of HD and ALC lymphomas include the expression of the CD30 molecule and of other activation antigens, and the inconsistent expression of T- or B-lineage antigens, often leading to a "null-cell" overall phenotype.[37,45,48,49] In addition, the expression of the intermediate-filament–associated protein restin,[50] and of the *c-kit* receptor protein,[51] has recently been shown to be restricted among human lymphomas to HD and ALC lymphomas. Such similarities, which also extend to "functional" parameters (such as the production of specific cytokines like interleukin-9[52]), further suggest that these two lymphoma entities are biologically related, and enhance diagnostic difficulties in distinguishing between them.[37]

In a comparative study[38] aimed at assessing in situ reactivity of a combination of antibodies such as CD30/BerH2, epithelial membrane antigen (EMA), CD15, and CD45, in addition to other antibodies suitable for paraffin tissues, a great proportion of ALC lymphoma cases expressed the CD30+, CD45+, CD15−, EMA− or + phenotypic profile, while in a comparable percentage of HD cases RS cells were found to express the CD30+, CD45−, CD15+, EMA− antigenic profile. However, since coexpression of CD30, CD45, and CD15 antigens was encountered in a far-from-negligible fraction of cases, the application of new discriminating markers would be extremely helpful. In this regard, recent studies[53-55] have demonstrated the great relevance of CD26 antigen expression in CD30+ ALC lymphomas[53] and have confirmed the consistent immunodetection of the CD40 molecule on RS cells of HD.[54,55] The results of a follow-up investigation into the complementary evidence deduced from the above-mentioned studies[53-55] have demonstrated that the combined application of anti-CD40 and anti-CD26 MoAbs does provide an additional significant aid in differentiating HD from CD30+ ALC lymphomas, because a CD26+, CD40− profile is restricted to the latter.[56]

GENOTYPING

Molecular genetic studies have attempted to detect the clonality and lineage of RS cells. Southern blot studies have detected both clonal rearrangements of immunoglobulin and T cell antigen receptor genes, with a predominance of clonal immunoglobulin heavy and/or light chain gene rearrangements.[57] However, these studies do not provide direct evidence to indicate that it is specifically the RS cells that are clonal and contain antigen receptor gene rearrangements.[35] Recently, clonal VDJ (variable diversity joining) rearrangements of the immunoglobulin heavy chain gene have been found in a subset of HD cases expressing B-lineage antigens by means of the polymerase chain reaction (PCR).[58] Furthermore, immunoglobulin heavy chain gene rearrangements have been detected in single RS cells in several cases by means of PCR studies combined with microdissection.[59] In nodular LP, there is also evidence for B cell clonality, as monotypic κ light chain restriction has

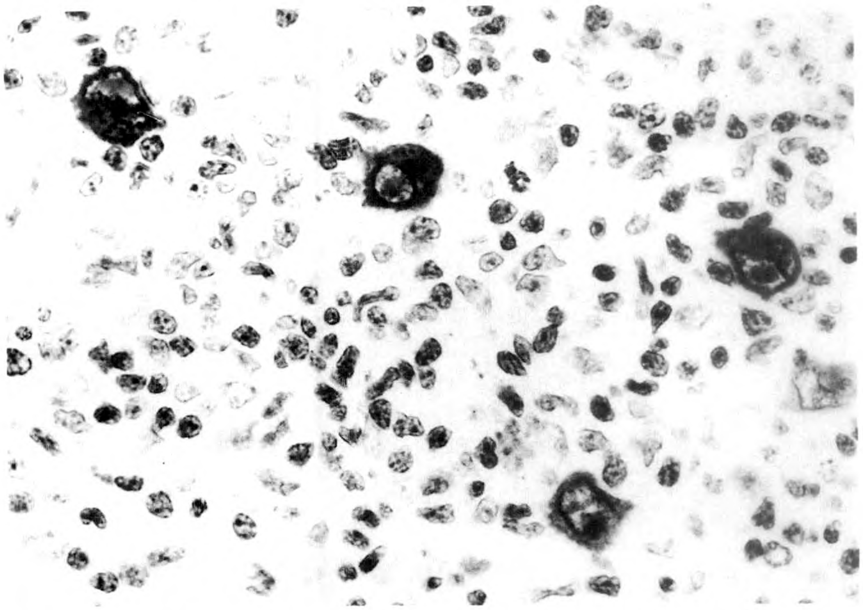

Fig 4. Hodgkin's disease, mixed cellularity subtype (Bouin-fixed, paraffin-embedded tissue section; APAAP method; hematoxylin counterstain; original ×630). Reed-Sternberg cells show strong cytoplasmic staining for EBV-encoded antigen LMP-1.

been demonstrated by immunohistochemical and in situ hybridization studies,[60,61] and clonality has been found in one case by means of a single PCR technique to analyze the immunoglobulin heavy chain gene.[59] However, one study found a polyclonal pattern of immunoglobulin gene rearrangements in LP by means of a single-cell PCR assay.[62]

EPSTEIN-BARR VIRUS ASSOCIATION

Hodgkin's disease shows epidemiologic features very similar to those of infectious disease. A bimodal age-incidence curve has been described, with an early peak comprising cases in young adults (15 to 34 years of age) and a second peak occurring in elderly individuals.[63] It has been suggested that HD in young adults may arise as an unusual and late host response to an infectious agent that is widespread in all communities. In the older HD patients, either the same virus may be reactivated from its latent state as a consequence of age-related decline in immunosurveillance, or these older cases may have a completely different etiopathogenesis. In addition to epidemiologic evidence, an association between EBV and HD has also been suggested by the findings of increased titers of EBV-induced antibodies in HD patients, and by an increased risk of developing HD after infectious mononucleosis.

A variety of different techniques at the DNA, RNA, and protein levels have been employed to analyze the presence of EBV in HD specimens. The first studies detected EBV-specific sequences in HD samples by Southern blotting and filter hybridization in about one third of the cases.[64,65] That the EBV genomes contained within the tumors were shown to be clonal indicates that the EBV-infected population had arisen from a single cell.[64-66] The PCR technique showed EBV DNA sequences in a considerably larger proportion of cases. However, that PCR positivity could have been derived from normal bystander cells is a possibility that cannot be ruled out. In situ hybridization (ISH; for either genomic DNA or EBV-encoded messenger RNAs such as EBERs [EBV-encoded small RNAs]) provided direct evidence that in EBV-positive cases the virus is prevalently found in RS cells and their precursors (Fig 3), further supporting the possible etiopathogenic role of the virus in HD.[66,67] Finally, immunostaining with antibodies against EBV-encoded proteins, such as LMP-1, was successfully used to detect EBV-positive cases (Fig 4) and also to ascertain that RS cells and their precursors harbor and actively transcribe the viral genome.[68] In a review of available data on the detection of EBV by different techniques, Drexler[35] reported that in a total of 928 HD cases investigated up to 1992, EBV positivity was around 19% by genomic DNA ISH, around 34% by either RNA ISH or Southern blot analysis of genomic DNA, around 45% by immunostaining with LMP-1, and around 56% by PCR amplification.

In a single-institution series comprising 188 HD cases (unpublished observations from the National Cancer Institute of Aviano, Italy) the association with EBV was investigated by ISH with an EBV RNA probe. Immunostaining for LMP-1 expression was also performed in 180 of these cases. The EBV positivity was around 27% by ISH and around 21% by immunostaining with LMP-1 on Bouin-fixed, paraffin-embedded tissue sections. Such discrepancies between the presence of EBV RNA detected by ISH and the absence of LMP-1 expression have already been reported. The lower positivity for LMP-1 by comparison with other studies may be attributed to the use of Bouin-fixed tissue sections. In this experience, a higher association of EBV was found with the MC HD subtype (27 of 42 cases or 64%) than with the other subtypes. An EBV positivity was detected in 18% of NS HD (21 of 115 cases), 29% of LD HD (2 of 7 cases), and 4% of LP HD (1 of 24 cases). These data are very similar to those found in two other European HD series.[68,69]

In conclusion, these data suggest that the EBV genome is primarily associated with the MC HD subset and may consequently further support the proposed subdivision of HD into two main groups, ie, the MC and NS subsets. The EBV is not generally found in the LP type.[60,67]

CONCLUSIONS

The histopathologic criteria of HD formulated by Lukes et al[2] have proved to be of invaluable utility to the diagnosis and study of HD. Today, immunohistochemical studies using a panel of antibodies directed against leukocyte antigens supplement the histologic criteria, and are particularly useful in the recognition of RS variants. Over the last 25 years, it has become evident that the nodular LP subtype may represent a distinct subset, and possibly a distinct disease entity, that can be distinguished from other types of HD by clinical, histologic, immunohistochemical, molecular biology, and virologic studies. Although the lineage of the RS cell in cases of NS and MC is still not clear, preliminary evidence suggests a possible B-cell lymphoid lineage. There is strong evidence for an association with EBV, particularly in MC cases.

REFERENCES

1. Lukes RJ, Craver LF, Hall TC, Rappaport H, Ruben P. Report of the Nomenclature Committee. Cancer Res 1966;26: 1311.

2. Lukes RJ, Butler JJ, Hicks EB. Natural history of Hodgkin's disease as related to its pathological picture. Cancer 1966;19:317-44.

3. Butler JJ, Pugh WC. Review of Hodgkin's disease. Hematol Pathol 1993;7:59-77.

4. Colby TV, Hoppe RT, Warnke RA. Hodgkin's disease: a clinicopathologic study of 659 cases. Cancer 1981;49:1848-58.

5. MacLennan KA, Bennet MH, Tu A, et al. Relationship of histopathologic features to survival and relapse in nodular sclerosing Hodgkin's disease. Cancer 1989;64:1686-93.

6. Anastasi J, Variakojis D. Heterogeneity in Hodgkin's disease: no simple answer for a complex disorder. Hum Pathol 1988;19:1251-4.

7. Chittal SM, Caverivière P, Schwarting R, et al. Monoclonal antibodies in the diagnosis of Hodgkin's disease: the search for a rational panel. Am J Surg Pathol 1988;12:9-21.

8. Carbone A. Hodgkin's disease in children. Tumori 1979; 65:719-28.

9. Kadin ME, Muramoto L, Said J. Expression of T-cell antigens on Reed-Sternberg cells in a subset of patients with nodular sclerosing and mixed cellularity Hodgkin's disease. Am J Pathol 1988;130:345-53.

10. Knowles DM II, Neri A, Pelicci PG, et al. Immunoglobulin and T-cell receptor b-chain gene rearrangement analysis of Hodgkin's disease: implications for lineage determination and differential diagnosis. Proc Natl Acad Sci USA 1986;83:7942-6.

11. Diebold J, Audoin J. Maladie de Hodgkin. Une ou plusieurs maladies? Ann Pathol 1989;9:86-91.

12. Kadin ME, Glatstein E, Dorfman RF. Clinicopathologic studies of 117 untreated patients subjected to laparotomy for the staging of Hodgkin's disease. Cancer 1971;27:1277-94.

13. Strum SB, Rappaport H. Interrelations of the histologic types of Hodgkin's disease. Arch Pathol 1971;91:127-34.

14. Pinkus GS, Said JW. Hodgkin's disease, lymphocyte predominance type, nodular — further evidence for a B-cell derivation. L & H variants of Reed-Sternberg cells express L26, a pan B cell marker. Am J Pathol 1988;133:211-7.

15. Timmens W, Visser L, Poppema S. Nodular lymphocyte predominance type of Hodgkin's disease is a germinal center lymphoma. Lab Invest 1986;54:457-61.

16. Kaplan HS. Hodgkin's disease. 2nd ed. Cambridge, Mass: Harvard University Press, 1980.

17. Jones SE, Fuks Z, Bull M, et al. Non-Hodgkin's lymphoma. Clinicopathologic correlation in 405 cases. Cancer 1973; 31:806-23.

18. Miettinen M, Franssila KO, Saxen E. Hodgkin's disease, lymphocyte predominance, nodular. Increased risk for subsequent non-Hodgkin's lymphomas. Cancer 1983;51:2293-300.

19. Dorfman RF. Relationship of histology to site in Hodgkin's disease. Cancer Res 1971;31:1786-93.

20. Harris NL, Jaffe ES, Stein H, et al. A revised European-American classification of lymphoid neoplasms: a proposal from the International Lymphoma Study Group. Blood 1994;84:1361-92.

21. Ahmed T, Wormser GP, Stahl RE, et al. Malignant lymphomas in a population at risk for acquired immune deficiency syndrome. Cancer 1987;60:719-23.

22. Hessol NA, Katz MH, Liu JY, Buchbinder SP, Rubino CJ, Holmberg SD. Increased incidence of Hodgkin's disease in homosexual men with HIV infection. Ann Intern Med 1992;117: 309-11.

23. Reynolds P, Saunders LD, Layefsky ME, Lemp GF. The spectrum of acquired immunodeficiency syndrome (AIDS)–associated malignancies in San Francisco, 1980-1987. Am J Epidemiol 1993;137:19-30.

24. Serraino D, Carbone A, Franceschi S, Tirelli U. Increased frequency of lymphocyte depletion and mixed cellularity subtypes of Hodgkin's disease in HIV infected patients. Eur J Cancer 1993;14:1948-50.

25. Andrieu JM, Roithmann S, Tourani JM, et al. Hodgkin's disease during HIV1 infection: the French Registry experience. Ann Oncol 1993;4:635-41.

26. Carbone A, Tirelli U, Vaccher E, et al. A clinicopathologic study of lymphoid neoplasias associated with human immunodeficiency virus infection in Italy. Cancer 1991;68:842-52.

27. Serrano M, Bellas C, Campo E, et al. Hodgkin's disease in patients with antibodies to human immunodeficiency virus: a study of 22 patients. Cancer 1990;65:2248-54.

28. Knowles DM, Chamulak GA, Subar M, et al. Lymphoid neoplasia associated with the acquired immunodeficiency syndrome (AIDS): the New York Medical Center experience with 105 patients (1981-1986). Ann Intern Med 1988;108:744-53.

29. Lowenthal DA, Straus DJ, Campbell SW, Gold JWM, Clarkson BD, Koziner B. AIDS-related lymphoid neoplasia. The Memorial Hospital experience. Cancer 1988;61:2325-37.

30. Rubio R. Hodgkin's disease associated with human immunodeficiency virus infection. Cancer 1994;73:2400-7.

31. Uccini S, Monaldo F, Ruco LP, et al. High frequency of Epstein-Barr virus genome in HIV-positive patients with Hodgkin's disease. Lancet 1989;i:1458.

32. Audouin J, Diebold J, Pallesen G. Frequent expression of Epstein-Barr virus latent membrane protein–1 in tumour cells of Hodgkin's disease in HIV-positive patients. J Pathol 1992;167: 381-4.

33. Herndier BG, Sanchez HC, Chang KL, Chen YY, Weiss LM. High prevalence of Epstein-Barr virus in the Reed-Sternberg cells of HIV-associated Hodgkin's disease. Am J Pathol 1993; 142:1073-9.

34. Tirelli U, Errante D, Dolcetti R, et al. Hodgkin's disease and HIV infection: clinicopathological features and virological features of 114 patients from the Italian Cooperative Group on AIDS and Tumors. J Clin Oncol 1995;13:1758-67.

35. Drexler HG. Recent results on the biology of Hodgkin and Reed-Sternberg cells. I. Biopsy material. Leuk Lymphoma 1992; 8:283-313.

36. Stein H. Ki-1 anaplastic large cell lymphoma: is it a discrete entity? Leuk Lymphoma 1993;10(suppl):81-4.

37. Kadin ME. Primary Ki-1–positive anaplastic large cell lymphoma: a distinct clinicopathologic entity. Ann Oncol 1994; 5(suppl 1):25-30.

38. Carbone A, Gloghini A, Volpe R. Paraffin section immunohistochemistry in the diagnosis of Hodgkin's disease and anaplastic large cell (CD30+) lymphomas. Virchows Arch [A] 1992;420:527-32.

39. Carbone A, Gloghini A, Volpe R, Boiocchi M. Antivimentin antibody reactivity with Reed-Sternberg cells of Hodgkin's disease. Virchows Arch [A] 1990;417:43-8.

40. Pinkus GS, Thomas P, Said JW. Leu-M1 — a marker for Reed-Sternberg cells in Hodgkin's disease. An immunoperoxidase study of paraffin-embedded tissues. Am J Pathol 1985;119:244-52.

41. Carbone A, Poletti A, Manconi R, Volpe R, Betta PG. Reliability of anti–Leu-M1 immunostaining in Hodgkin's disease. Arch Pathol Lab Med 1986;110:465-6.

42. Carbone A, Manconi R, Poletti A, et al. Reed-Sternberg cells and their cell microenvironment in Hodgkin's disease with reference to macrophage-histiocytes and interdigitating reticulum cells. Cancer 1987;60:2662-8.

43. Carbone A, Gloghini A, Gattei V, et al. Expression of functional CD40 antigen on Reed-Sternberg cells and Hodgkin's disease cell lines. Blood 1995;85:780-9.

44. Stein H, Mason DY, Gerdes J, et al. The expression of the Hodgkin's disease associated antigen Ki-1 in reactive and neoplastic lymphoid tissue: evidence that Reed-Sternberg cells and histiocytic malignancies are derived from activated lymphoid cells. Blood 1985;66:848-58.

45. Agnarsson BA, Kadin ME. Ki-1 positive large cell lymphoma: a morphologic and immunologic study of 19 cases. Am J Surg Pathol 1988;12:264-74.

46. Hall PA, D'Ardenne AJ, Stansfeld AG. Paraffin section immunohistochemistry. II. Hodgkin's disease and large cell anaplastic (Ki1) lymphoma. Histopathology 1988;13:161-9.

47. Rosso R, Paulli M, Magrini U, et al. Anaplastic large cell lymphoma, CD30/Ki-1 positive, expressing the CD15/Leu-M1 antigen: immunohistochemical and morphological relationships to Hodgkin's disease. Virchows Arch [A] 1990;416:229-35.

48. Frizzera G. The distinction of Hodgkin's disease from anaplastic large cell lymphoma. Semin Diagn Pathol 1992;9:291-6.

49. Carbone A, Gloghini A, Volpe R. Immunohistochemistry of Hodgkin and non-Hodgkin lymphomas with emphasis on the diagnostic significance of the BNH9 antibody reactivity with anaplastic large cell (CD30 positive) lymphomas. Cancer 1992; 70:2691-8.

50. Delabie J, Shipman R, Brüggen J, et al. Expression of the novel intermediate filament-associated protein restin in Hodgkin's disease and anaplastic large-cell lymphoma. Blood 1992;80:2891-6.

51. Pinto A, Gloghini A, Gattei V, Aldinucci D, Zagonel V, Carbone A. Expression of the *c-kit* receptor in human lymphomas is restricted to Hodgkin's disease and CD30$^+$ anaplastic large cell lymphomas. Blood 1994;83:785-92.

52. Merz H, Houssiau FA, Orscheschek K, et al. Interleukin-9 expression in human malignant lymphomas: unique association with Hodgkin's disease and large cell anaplastic lymphoma. Blood 1991;78:1311-7.

53. Carbone A, Cozzi M, Gloghini A, Pinto A. CD26/DPPIV expression in human lymphomas is restricted to CD30-positive anaplastic large cell and a subset of T-cell non-Hodgkin's lymphomas. Hum Pathol 1994;25:1360-5.

54. Carbone A, Gloghini A, Gruss H-J, Pinto A. CD40 antigen expression on Reed-Sternberg cells. A reliable diagnostic tool for Hodgkin's disease. Am J Pathol 1995;146:780-1.

55. Murray PG, Oates J, Reynolds GM, Crocker L, Young LS. Expression of B7 (CD80) and CD40 antigens and the CD40 ligand in Hodgkin's disease is independent of latent Epstein-Barr virus infection. J Clin Pathol Mol Pathol 1995;48:M105-M108.

56. Carbone A, Gloghini A, Zagonel V, Pinto A. An update on the diagnostic relevance of CD26 antigen expression in CD30 positive anaplastic large cell lymphomas. Hum Pathol 1995;26: 1169-70.

57. Weiss LM, Strickler JG, Hu E, Warnke RA, Sklar J. Immunoglobulin gene rearrangements in tissues involved by Hodgkin's disease. Hum Pathol 1986;17:1006-14.

58. Tamaru J, Hummel M, Zemlin M, Kalvelage B, Stein H. Hodgkin's disease with a B-cell phenotype often shows a VDJ rearrangement and somatic mutations in the V genes. Blood 1994;84:708-15.

59. Kuppers R, Rajewsky K, Zhao M, et al. Hodgkin disease: Hodgkin and Reed-Sternberg cells picked from histological sections show clonal immunoglobulin gene rearrangements and appear to be derived from B cells at various stages of development. Proc Natl Acad Sci USA 1994;91:10962-6.

60. Schmid C, Sargent C, Isaacson P. L & H cells of nodular lymphocyte predominant Hodgkin's disease show immunoglobu-

lin light-chain restriction. Am J Pathol 1991;139:1281-9.

61. Stoler MH, Nichols GE, Symbula M, Weiss LM. Nodular L & H lymphocyte predominance Hodgkin's disease: evidence for a kappa light chain–restricted monotypic B cell neoplasm. Am J Pathol 1995;146:812-8.

62. Delabie J, Tierens A, Wu G, Weisenburger DD, Chan WC. Lymphocyte predominance Hodgkin's disease: lineage and clonality determination using a single-cell assay. Blood 1994;84:3291-8.

63. Gutensohn NM, Cole P. Epidemiology of Hodgkin's disease. Semin Oncol 1980;7:92-102.

64. Anagnostopoulos I, Herbst H, Niedobitek G, Stein H. Demonstration of monoclonal EBV genomes in Hodgkin's disease and Ki-1–positive anaplastic large cell lymphoma by combined Southern blot and in situ hybridization. Blood 1989;74:810-6.

65. Weiss LM, Strickler JG, Warnke RA, Purtilo DT, Sklar J. Epstein-Barr viral DNA in tissues of Hodgkin's disease. Am J Pathol 1987;129:86-91.

66. Weiss LM, Movahed LA, Warnke RA, Sklar J. Detection of Epstein-Barr viral genomes in Reed Sternberg cells of Hodgkin's disease. N Engl J Med 1989;320:502-6.

67. Weiss LM, Chen Y-Y, Liu X-F, Shibata D. Epstein-Barr virus and Hodgkin's disease: a correlative in situ hybridization and polymerase chain reaction study. Am J Pathol 1991;139:1259-65.

68. Pallesen G, Hamilton-Dutoit SJ, Rowe M, Young LS. Expression of Epstein-Barr virus latent gene products in tumor cells of Hodgkin's disease. Lancet 1991;337:320-2.

69. Khan G, Norton AJ, Slavin G. Epstein-Barr virus in Hodgkin disease. Relation to age and subtype. Cancer 1993;71:3124-9.

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